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Polymer-Supported Mitsunobu Ether Formation and its Use in Combinatorial Chemistry

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Abstract: Aromatic hydroxy acids have been attached to a polymeric solid support and the phenolic hydroxy groups have been reacted with a variety of primary and secondary alcohols under the conditions of the Mitsunobu reaction (triphenylphosphine and diethyl azodicarboxylate) in tetrahydrofuran. In most cases the reaction provided a nearly quantitative yield of alkyl aryl ethers, as determined after cleaving the product from the resin. To demonstrate that the polymer-supported Mitsunobu reaction is useful for combinatorial library synthesis, we synthesized a number of model compounds and a simple three randomization step library composed of 4,200 different compounds.

To create a diversity of structural types within a combinatorial library, one needs a collection of organic reactions that can be performed on solid phase (for a recent reviews on library techniques see, e.g.[1,2]). There are several criteria for the selection of suitable chemistries for use in library design and synthesis. Relevant features we consider are (i) yield and purity of products, (ii) variety of available building blocks, (iii) compatibility with other chemistries, and (iv) "user friendly" reaction conditions. Out of the myriad of organic reactions, we selected the Mitsunobu ether formation[3] as a suitable reaction for combinatorial chemistry according to the above criteria. Out of two possible etherification modes we have studied the reaction of polymer-supported phenol with alcohol in solution. The "reverse" manner of this reaction with resin-bound alcohol for attaching Tyr to the resin has recently been described.[4]

Since the polymer-supported etherification can also be a very useful method for modification of Tyr residues in peptides, we used N-acetylated tyrosine on polyethyleneglycol grafted polystyrene-divinylbenzene copolymer TentaGel S OH (TG, Rapp Polymere, Germany) as a model compound[5] to optimize the reaction conditions with respect to solvent, ratio and excess of reagents (i.e. alcohol, triphenylphosphine (PPh_3) and diethyl azodicarboxylate (DEAD)), temperature and reaction time (Scheme 1).

The following procedure was found generally acceptable. The reaction was carried out in a polypropylene syringe equipped with a polypropylene frit.[6] Ac-Tyr-O-TG (0.2 - 0.3 ml, substitution ca 0.2 mmol/g) was washed five times with dry tetrahydrofurane (THF). One-half ml of a 1 M solution of PPh_3 (262 mg/ml) in THF and 1 mmol of alcohol was added and the slurry shaken. Then, 0.5 mmol (78 µl) of DEAD was diluted by THF (234 µl) and this solution was added to the resin in four portions at 5 min intervals and the mixture was placed on a tumbler. After one hour, the resin was washed five times with DMF and three times with methanol.

Scheme 1. Ether formation on phenolic hydroxy groups of resin bound Ac-Tyr.

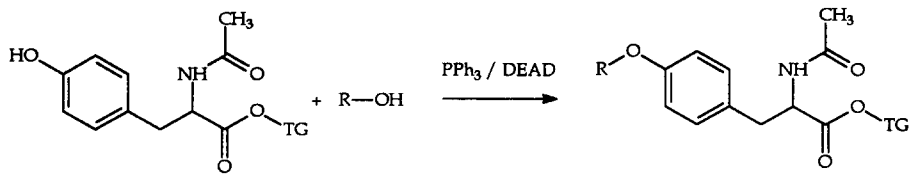


Table 1. Alkyl Aryl Ethers Prepared by Polymer-Supported Mitsunobu Reaction

Alcohol	Alkyl ether		Retention time		Ethyl ether	
	A	B	A	B	A	B
Methanol	95%	99%	19.6	17.5	< 1%	< 1%
Ethanol	96%	99%	22.2	20.5	main product	
2-Propanol	93%	99%	23.2	22.8	< 1%	< 1%
1-Butanol	75%	80%	27.3	27.1	23%	20%
Allyl alcohol	95%	99%	23.0	22.4	< 1%	< 1%
1,3-Propanediol	94%	99%	17.8	16.7	< 1%	< 1%
Benzyl alcohol	99%	93%	27.8	27.3	< 1%	< 1%
4-Methoxybenzyl alcohol	98%	99%	27.6	27.4	< 1%	< 1%
4-(Methylmercapto)benzyl alcohol	78%	90%	30.3	29.0	< 1%	< 1%
2-(Hydroxymethyl)furan	66%	74%	25.3	23.9	9 %	8 %
3-(Hydroxymethyl)furan	98%	99%	25.3	23.9	< 1%	< 1%
2-(Hydroxymethyl)thiophene	94%	93%	27.3	26.2	2 %	< 1%
4-Methyl-5-(2-hydroxyethyl)thiazole	59%	85 (96)%	19.6	17.8	2 %	13 (<1)%
2-(Hydroxymethyl)pyridine	84%	99%	16.3	15.1	6 %	< 1%
3-(Hydroxymethyl)pyridine	86%	99%	16.2	14.9	6 %	< 1%
4-(Hydroxymethyl)pyridine	57%	92%	16.2	15.2	6 %	< 1%
2,6-Di(hydroxymethyl)pyridine	82%	98%	16.0	15.5	9 %	< 1 %
1-(2-Hydroxyethyl)pyrrolidine	43 (60)%	53 (82)%	15.5	14.2	5 (<1)%	8 (<1)%
1-(2-Hydroxyethyl)-2-pyrrolidinone	42%	47 (85)%	19.5	18.0	10 %	47 (9)%
2-(Boc-amino)ethyl alcohol	39 (52)%	51 (82)%	25.6	24.5	46 (29)%	45 (15)%
3-(Fmoc-amino)propyl alcohol	90%	69%	15.1	13.7	5 %	30 %

Note: Entries "A" and "B" refer to O-alkylated N-acetyl tyrosine and 4-hydroxybenzoyl-glycine, respectively. Analytical gradient HPLC profile was run on a Protein & Peptide C18 4x250 mm analytical column (Vydac), gradient 0 - 60 % of ACN in 30 min, retention time of N-acetyl tyrosine and 4-hydroxybenzoyl-glycine was 14.4 and 13.4 min, respectively. The correct molecular weight was confirmed by mass spectrometry (PE-Sciex API III+ with an articulated ion spray sample inlet system). The yield of alkyl ethers and the side-product, ethyl ether, was estimated from the analytical HPLC profile at 280 nm. The amount of isopropyl ether, detected when using diisopropyl azodicarboxylate, is given in parenthesis. t-Butanol, (1R)-(-)-nopol, 1-(hydroxymethyl)-3-methyl-2-norbornane, 1-(hydroxymethyl)-3,5-dimethylpyrazole, and 9-(hydroxymethyl)fluorene did not provide satisfactory results, even after reacting overnight (yield < 10%).

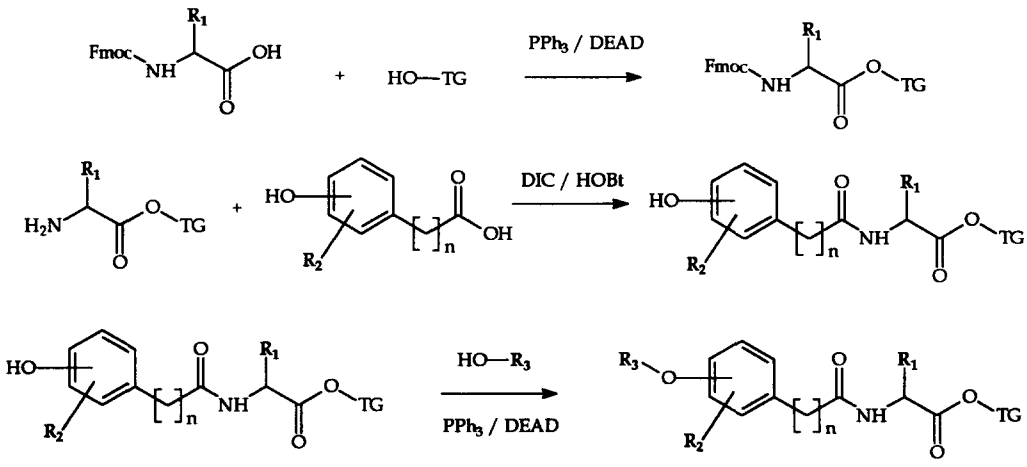
The product was cleaved from the resin by exposure to 0.5 ml of 0.5 % NaOH for 10 min., acidified with dilute acetic acid, and injected on an analytical HPLC. A variety of primary and secondary alcohols including aliphatic, aromatic and heterocyclic have been tested (for results see Table 1, entries "A").

The yield and purity of the product is sensitive to elevated temperature, thus, even when working in small scale (ca 0.2 ml of swollen resin), DEAD must be added slowly. To accommodate this reaction for the synthesis of libraries, we prefer to dilute DEAD in THF and add the solution in several portions. Fast addition of undiluted DEAD resulted in the formation of a side-product, the corresponding ethyl ether. Increased temperature caused the decomposition of DEAD and the liberated ethyl alcohol was activated by the remaining DEAD and reacted with phenolic hydroxy groups. Cooling the reaction mixture during DEAD addition is necessary when working in larger scale. Nevertheless, in the case of some alcohols, the formation of ethyl ether was substantial, even when the reaction mixture was cooled to -15 °C before the addition of DEAD. The extent of ethyl ether formation is shown in Table 1. In the case of these alcohols, the use of diisopropyl azodicarboxylate was superior to DEAD. While the reaction time is longer (typically 3 h), the quantity of side-product, diisopropyl ether, was reduced to an acceptable level.

We did not observe satisfactory results when coupling was performed in N-methyl morpholine, reportedly the solvent of choice for the reaction of polymer-supported alcohols and phenols in solution.[4]

Polymer-supported Mitsunobu ether formation can be used in the design and synthesis of combinatorial libraries in many different ways. We incorporated this reaction into the one-bead-one-compound combinatorial library strategy,[7] using the split-mix method[8] for library synthesis. Our model library involved three randomization steps (see Scheme 2): (i) attachment of N-protected amino acids to the polymeric support via an ester bond, (ii) coupling of aromatic hydroxy acids, and (iii) Mitsunobu ether formation using a set of alcohols.

Scheme 2. Model library including three randomization steps.



Only N-alpha amino acids are shown on Scheme 2, however, any amino acid can be used in this step. The amino groups of the amino acids were used to react with an aromatic hydroxy acid. Since the activated carboxyl group can acylate unprotected hydroxy groups, we prepared as a model 4-hydroxybenzoyl-Gly-OH, using both unprotected and tert-butyl protected 4-hydroxybenzoic acid (DIC/HOBt activation and 1 h reaction time). Both products provided the same gradient HPLC profile, showing no sign of undesirable acylation of unprotected hydroxy groups under these conditions.

Before the library was synthesized, we tested the same set of alcohols on the model compound: p-hydroxybenzoyl-Gly-O-TG. The results are summarized in Table 1, entries "B". We then selected a representative set of five amino acids (Gly, Ser, Pro, Glu, and Arg) and five aromatic hydroxy acids (2- and 3-hydroxybenzoic acids, 3-methoxy(and nitro)benzoic acid, 4-hydroxycinnamic acid, and 2-hydroxynicotinic acid) and tested them with two alcohols, i-propanol and benzyl alcohol. Representative results with Gly as the selected amino acid are shown in Table 2, all products have been characterized by mass spectra and ^{1}H NMR spectra.

Finaly, a small model library containing 4,200 compounds, composed of 20 natural amino acids, 10 aromatic hydroxy acids, and 21 alcohols have been prepared. Structure determination and results of on-bead binding assay for model targets will be described elsewhere.

Table 2. Etherification of Phenolic Hydroxy Group by i-Propanol (A) and Benzyl Alcohol (B)

Hydroxy Acid	Isolated yield		Retention time	
	A	B	A	B
4-Hydroxybenzoic acid	69 %	60 %	22.8	27.8
2-Hydroxyphenylacetic acid	90 %	95 %	24.9	29.2
4-Hydroxyphenylacetic acid	83 %	81 %	23.2	27.8
4-Hydroxyphenylpropionic acid	81 %	87 %	23.7	27.3

Polymer-supported Mitsunobu etherification of resin bound phenolic groups was shown to be a very useful reaction for creating chemical diversity in synthetic combinatorial libraries. The reaction provides almost quantitative yields with a variety of alcohols. The reaction conditions are "user friendly" and, last but not least, there are a vast number of alcohols that are available commercially.

References and notes

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