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Solid-Phase Synthesis of β -Hydroxy Ketones Via DNA-Compatible Organocatalytic Aldol Reactions

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Abstract

One-bead-one-compound (OBOC) libraries constructed by solid-phase split-and-pool synthesis are a valuable source of protein ligands. Most OBOC libraries are comprised of oligoamides, particularly peptides, peptoids and peptoid-inspired molecules. Further diversification of the chemical space covered by OBOC libraries is desirable. Towards this end, we report here that the proline-catalyzed asymmetric aldol reaction, developed by List and Barbas for solution-phase synthesis, also works well for coupling immobilized aldehydes and soluble ketones. These reaction conditions do not compromise the amplification of DNA by the polymerase chain reaction. Thus, this chemistry should be useful for the construction of novel DNA-encoded OBOC libraries by solid-phase synthesis.

Graphical Abstract

Keywords

Aldol reaction; solid-phase synthesis; organocatalysis; one bead one compound libraries; DNA-encoded libraries

Introduction

The creation of one bead one compound (OBOC) libraries by split and pool solid-phase synthesis^{1, 2} is a powerful method for the rapid and inexpensive synthesis of large libraries of potential protein ligands.^{3–5} When created on a suitable resin, such as TentaGel, these libraries can be screened by incubation with a labeled protein and manually picking beads that retain a high level of fluorescence.⁶ The compound is released from the bead and its structure characterized, usually by mass spectrometry (MS).^{7, 8} While the utility of this

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approach to the discovery of protein ligands had previously been limited by a number of nagging technical problems, most of these issues have now been addressed. For example, bead screening has a notoriously high false positive rate, but it has been shown that if one screens redundant libraries, hits that are isolated on multiple beads are almost always *bona fide* ligands.⁹

An important advance in OBOC technology was the application of encoding¹⁰ tags to the beads.^{11, 12} This eliminates the need for direct MS-based characterization of hit compounds, which had largely limited OBOC libraries to oligomers that fragment in a logical fashion in the tandem MS or can be sequenced by Edman degradation, such as peptides and peptoids.^{3, 13–15} DNA-encoded OBOC libraries, in particular, confer the additional advantage that the library can be constructed and screened on 10 μ m TentaGel beads, which are small enough to pass through a flow cytometer. Isolation of hit beads by using a fluorescent activated cell sorter (FACS) is far more convenient than manual picking and facilitates two-color screens that demand selectivity for a target over one or more off-targets.¹⁶ The encoding tags are then deep sequenced.

With this powerful technology now established, it is important to expand the repertoire of chemical reactions that can be used to make OBOC libraries. Reactions that create chiral centers are of particular interest, since there is a general feeling in the screening community that molecules with a high level of “three-dimensionality” and natural product-like character would tend to bind their targets with higher selectivity than the flat, largely aromatic compounds that dominate most current screening collections.^{17–20} Towards this end, we report here conditions for the efficient solid-phase synthesis of β -hydroxy ketones via the organocatalytic aldol reaction^{21–28} of immobilized aldehydes and soluble ketones (Scheme 1C). Another important consideration in developing reactions for the synthesis of DNA-encoded libraries is to evaluate the level of chemical damage suffered by the encoding DNA that would compromise its amplifiability. For example, routine amino acid or peptoid couplings are known to degrade encoding tags to some degree (Scheme 1 A and B).²⁹ The mild conditions for the aldol reaction are shown to be quite compatible with the presence of the DNA tags. Thus, this chemistry should be useful in the synthesis of novel DNA-encoded OBOC libraries.¹²

Results and Discussion

TentaGel HL NH₂ resin (110 μ m, 0.46 mmol/g) was modified with a disulfide-containing linker to allow the release of compounds from the solid support under mild reductive conditions (treatment with TCEP) to avoid potential dehydration following the aldol addition product under the usual acidic cleavage conditions. The amino terminal end of the linker was acylated with 4-formyl benzoic acid using Oxyma coupling conditions³⁰ (see supporting information). Various solvents and conditions were then explored for the (*S*)-proline-catalyzed addition of acetone to this immobilized aldehyde. We included in all of the reactions beads that contained an inactive control substrate made by coupling benzoic acid (with no formyl group) to the linker. This inert compound served as an internal standard to aid in the quantification of the conversion of the formyl-containing substrate.

We found using DMSO as the solvent and incubating for 16 hours at room temperature in the presence of one equivalent of proline (with respect to the aldehyde on the resin) provided the highest yield of the desired aldol product (Table 1, entry 6), as determined by liquid chromatography-mass spectrometry (LC-MS). This result was obtained using a large excess of ketone with respect to the immobilized aldehyde (100:1 molar ratio). Reactions employing lower ketone levels resulted in poor conversion. Poor results were also obtained when the amount of proline was reduced. Note that proline was used as a suspension due to its modest solubility in all of the solvents examined. We also examined various proline derivatives such as tetrazole substituted-proline³¹ 2-(methoxymethyl)-pyrrolidine²³ and prolineamide,²³ but found that (*S*)-proline was the best choice (Table 2), which was also true for the solution-phase organocatalytic aldol reaction.²³

The aldol product was formed in a modest 73% e.e. The use of a variety of additives such as chiral diols,³² thioureas³³ and guanidinium salts³⁴ or lowering the temperature (4°C and –10°C) did not improve the enantioselectivity significantly (data not shown). Hence, the conditions described above were used to explore the scope of the reaction with respect to the aldehyde and ketone components.

Table 3 shows the results of the reaction of acetone with a number of immobilized aldehydes. All of the reactions proceeded with almost quantitative conversion, even when an ortho-substituent was present (**1g**, **1h**). However, when the formyl group was ortho to the amide no reaction was observed (data not shown). A heteroaromatic aldehyde also worked well (**1i**). In general, the enantioselectivities were modest, with e.e. ranging from 54%–79%. A notable exception was **1f**, which was produced as a single stereoisomer. This is typical for the solution phase aldol reaction of benzaldehydes with electron-withdrawing substituents ortho to the aldehyde, as noted by List and co-workers.³⁵

Next, direct aldol reactions between the p-formyl benzoic acid-derived immobilized substrate and various soluble ketones were investigated (Table 4). The reactivity of most soluble ketones was excellent (90%–99% conversion), with the sole exception of dimethoxyacetone, which was a respectable 78%. In all cases, the anti product was favored, generally providing drs of 3:1 – 4:1. Hydroxyacetone was exceptional in that no detectable syn product was formed using this ketone. The enantioselectivity was improved over the reactions employing acetone. With the exception of cyclopentanone (54% e.e.), the major anti product was produced in 84%–98% e.e. The use of nonsymmetrical ketones produced mixtures of regioisomers. For 2-butanone, there was no preference, with both regioisomers produced in equal amounts. However, for hydroxyacetone and methoxyacetone, the regioisomer resulting from the more substituted carbon acting as the nucleophile (called the non-terminal product in Table 4) was strongly favored (>20:1). These results largely mirror those obtained in the solution phase aldol reaction.³⁶

With an eye towards incorporating useful functionality into the aldol products, Fmoc-protected 1-amino-2-propanone and Fmoc-protected 4-piperidinone were examined as substrates, but these reactions failed to produce the desired aldol products. This is probably due to the lower solubility of these ketones in DMSO. The same high concentration that could be achieved for the ketones shown in Table 4 was not possible with these substrates.

This is likely to be a problem using any ketone containing an Fmoc-protected amine. Therefore, we turned our attention to trying to incorporate an azido moiety into the aldol product. An azide can be further elaborated using copper-catalyzed Huisgen cycloadditions, aza-Wittig reactions or can be reduced to an amine with a phosphine. Unfortunately, under the standard conditions described above, azidoacetone provided a poor yield of the desired aldol product. However, we found that the addition of a guanidinium-type ionic liquid (see SI for a detailed procedure), as reported by Martinez-Casteneda, et al.,³⁷ provided the desired α -azido- β -hydroxy ketone in excellent yield as well as with high stereo-, regio-, and enantioselectivity (Fig 1A). However, a two-day incubation at 4°C was required to obtain this result. Thus, we also examined the possibility of displacing the alcohol in the aldol product with azide under Mitsunobu conditions (Fig 1B). After several attempts, we found that using a large excess (50 equivalents) of all the soluble reagents required for the Mitsunobu reaction provided a high yield of the desired product (see SI for a detailed procedure). Thus, using either of these protocols, a versatile azido group can be incorporated into the aldol product, allowing further elaboration of the molecule.

We hypothesized that the mild conditions employed for the proline-catalyzed aldol reaction would be compatible with encoding DNA. To address this point, we employed a quantitative polymerase chain reaction (qPCR) assay described by Malone and Paegel²⁹ in which a 200 bp double-stranded DNA molecule is subjected to the reaction conditions and the amount of DNA that remains amplifiable by the polymerase chain reaction (PCR) is quantified. Thus, the immobilized DNA was incubated for 16 hours at room temperature in the presence of acetone, immobilized aldehyde and proline. The qPCR data showed that at least 90% of the DNA could be amplified relative to a control reaction in which the DNA was not exposed to any chemical reagents (Fig. 2).

In conclusion, the proline-catalyzed aldol reaction has been adapted to bead-based synthesis. To a large degree, the trends observed in the solid-phase reaction parallel those observed when the reaction is carried out in solution, the major difference being the need for a large excess of ketone and an equivalent of proline. As anticipated, the reaction conditions have little or no effect on the amplifiability of encoding DNA. Therefore, this process should be a valuable tool for the construction of DNA-encoded OBOC libraries. Finally, we have employed in this study a disulfide linker that can be cleaved reductively³⁸ to ensure that our analysis of the intrinsic stereoselectivity of the aldol reaction was not compromised by the chemistry used to release the compound from the bead, as might be the case for acidic cleavage, for example. In subsequent work we have found that cleavage of compounds from resins with RAM linkers by brief treatment with dilute trifluoroacetic acid does not result in detectable racemization (data not shown), so there is no need to use the disulfide linker if this is not convenient.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Incorporation of azide group to aldol product (A) aldol reaction using azido acetone. Diastereoisomeric and regioisomeric ratios were obtained by ¹H NMR of crude mixture. ee were determined by chiral HPLC. (B) Convert hydroxy group to azide using Mitsunobu reaction.

Figure 2. Quantification of amplifiable DNA remaining ratio by DNA damage assay. (A) Calibration curve made by DNA standard samples of a known concentration. $R=0.96$ (B) Remaining DNA damage ratio of aldol reaction and mitsunobu reaction. The remaining DNA ratio of aldol reaction values were $91 \pm 14\%$. That of mitsunobu reaction were $93 \pm 8\%$. Results were generated from triplicate experiments.

(A) Previous work: Fmoc-SPPS **(B) Previous work: Peptoid construction using 'submonomer' synthesis** **(C) This work: Proline catalyzed Direct aldol reaction on the solid support** **Scheme 1.**

Chemistry suitable for the construction of one bead one compound libraries by solid-phase synthesis. The red stars on the DNA indicate chemical modifications that would impede amplification of the encoding tag.

Table 1.

Establishment of optimal conditions.

Entry	Solvent	Temp (°C)	Conversion (%)
1	acetonitrile	4	0
2	acetonitrile	20	2
3	NMP	4	11
4	NMP	20	42
5	DMSO	4	75
6	DMSO	20	95
7	DMF	4	4
8	DMF	20	35

Reaction conditions: Reactions were carried out using 1 eq. of (*S*)-proline (relative to the immobilized aldehyde (0.006 mmol)) and acetone (100 eq, 44 μ L) in 120 μ L DMSO. Conversion was determined from cleaved crude mixture by monitoring LC-MS analysis of the crude product after reductive cleavage from the beads. This was calculated by measuring the ratio of the remaining aldehyde peak and that of an internal standard (the equivalent molecule lacking a formyl group) (see supporting info).

Table 2.

Comparison of various proline-derived catalysts.

Entry	catalyst	amount of catalyst (eq)	Conversion (%)	ee (%)
1		1	99	73
2 ^a		1	99	70
3		5	98	8
4		3	99	1

Reaction conditions: Reactions were carried out using organocatalyst and acetone (100 eq, 44 μ L) to resin loading (0.006 mmol) in 120 μ L of DMSO for 16 hours. Conversion was determined from cleaved crude mixture by monitoring LC-MS, calculated by two peak ratio between starting aldehyde and inactive benzoic ring which is used for internal standard. (see supporting info)

^aReaction was completed in 4h.

Table 3.

Substrate scope of acceptor aldehyde.

Reaction conditions: Reactions were carried out using 1 eq (S)-proline and acetone (100 eq, 44 μ L) to resin loading (0.006 mmol) in 120 μ L DMSO. Conversion was determined from cleaved crude mixture by monitoring LC-MS, calculated by two peak ratio between starting aldehyde and inactive benzoic ring which is used for internal standard. Ee were determined by chiral HPLC (see supporting info).

^aTo monitor products by UV length of 254 nm, Fmoc-4-Abz-OH was coupled before coupling 5-formylthiophen-2-carboxylic acid.

Table 4.

Substrate scope of donor ketone

Entry	Ketone	Conversion (<i>anti-2</i> + <i>syn-2</i> + <i>3</i>) (%)	2		rr (2 : 3)	3
			dr (<i>anti</i> : <i>syn</i>)	ee (%) <i>anti</i> ; (<i>syn</i>)		ee (%)
1	cyclopentanone	99 (<i>anti-2a</i> + <i>syn-2a</i>)	4:1	57 (69)	–	–
2	cyclohexanone	90 (<i>anti-2b</i> + <i>syn-2b</i>)	1:1	85 (80)	–	–
3	dimethoxyacetone	78 (<i>anti-2c</i> + <i>syn-2c</i>)	4:1	84 (n.d.) ^a	–	–
4	methoxyacetone	90 (<i>anti-2d</i> + <i>syn-2d</i> + trace 3d)	3:1	98 (n.d.) ^b	> 20:1	n.d. ^b
5	2-butanone	99 (<i>anti-2e</i> + <i>syn-2e</i> + 3e)	4:1	96 (80)	1:1	94
6	hydroxyacetone	99 (<i>anti-2f</i> + <i>syn-2f</i> + trace 3f)	> 20:1	84 (n.d.) ^b	> 20:1	n.d. ^b

Reaction conditions: Reactions were carried out using 1 eq (S)-proline and 100 eq of ketones to resin loading (0.006 mmol) in 120 μ L DMSO. Conversion were determined from cleaved crude mixture by monitoring LC-MS, calculated by two peak ratio between starting aldehyde and inactive benzoic ring which is used for internal standard. ee were determined by chiral HPLC. Diastereoisomeric and regioisomeric ratios were obtained by ¹H NMR of crude mixture.

^aNot determined because chiral separation was failed.

^bNot determined because we could not obtain enough amount of compound for analysis.