

Figure 2. The signal corresponding to the quadrant aromatic stretches of the labeled oligonucleotides, as two single samples and in a mixture.

separation and in varying proportions. In addition, these initial experiments indicate that oligonucleotide probe design is crucial and there is considerable potential for further development particularly as both nonfluorescent and fluorescent chromophores can be used as SERRS labels. The modified oligonucleotide probes can also still be used as substrates for polymerases, therefore demonstrating that they remain biologically active. These results prove that, by considering the chemistry involved, SERRS can feature in the development of more effective DNA assays allowing the development of assay formats not possible by conventional methods.

Experimental Section

A solution of modified oligonucleotide (20 μL , $4 \times 10^{-8} \text{ M}$) was premixed on ice with an aliquot of spermine tetrahydrochloride (20 μL , $8 \times 10^{-2} \text{ M}$). Water (500 μL) and citrate-reduced silver colloid (500 μL) were added to this solution. Analysis used a Renishaw 2000 Raman Microprobe with excitation provided by a Spectra-Physics Model 2020 argon-ion laser (100 mW, $\lambda = 514.5 \text{ nm}$). The detector was a charge-coupled device (CCD). Samples were analysed over 1 s in a plastic micro-titre plate using a X10 objective with the grating centred at 1400 cm^{-1} .

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α -Oxymethyl Ketone Enolates for the Asymmetric Mannich Reaction. From Acetylene and *N*-Alkoxy carbonylimines to β -Amino Acids**

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The reactions of enolizable carbonyl compounds with azomethine functions, usually referred to as Mannich-type reactions (also termed aza-aldol reactions), result in the formation of β -amino acids, ketones, or aldehydes.^[1] These reactions are conceptually equivalent to the aldol reactions, but, in sharp contrast, they have been substantially less developed.^[2, 3, 4] There are two reasons that can justify this situation: firstly, the poorer electrophilicity of the azomethine function relative to that of the carbonyl function, and secondly, the preference of enolizable azomethines to undergo α -deprotonation rather than addition.^[5] To date, there are two main ways to approach these problems. One strategy lies in the use of activated forms of azomethines,^[1, 2] and the other in the use of trialkylsilylenol ethers or *O*-(trialkylsilyl)ketene acetals as nucleophiles.^[3, 6] The later strategy has led to outstanding advances in enantioselective Mannich reactions^[7] catalyzed by chiral ruthenium,^[8a-b] palladium,^[8c-e] and copper^[8e-g] complexes. Some highly diastereoselective methods involving chiral azomethines are also known,^[6, 9] but in most

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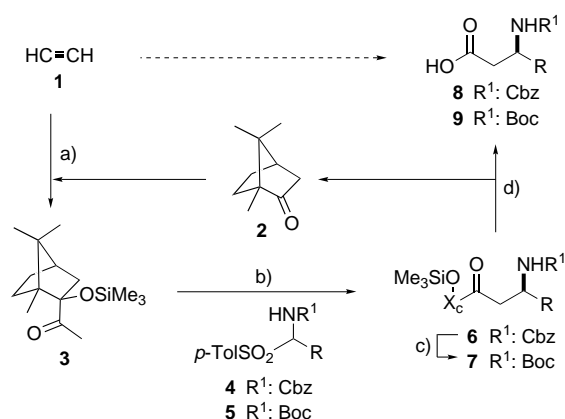
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instances, the chiral auxiliary is often difficult to recover. Despite these achievements, however, there are very few known diastereoselective methods involving chiral enolates,^[1] which are almost paramount in the parent aldol reactions. Most importantly, the stereoselectivity attained with the hitherto known α -unsubstituted chiral enolates is still rather inadequate.^[10, 11] We report here on a new diastereoselective Mannich-type reaction that helps to solve the above problems.

The lithium enolate of the methyl ketone **3**, the latter being readily prepared from acetylene (**1**) and (1*R*)-(+)-camphor (**2**),^[12] reacted, in the presence of an excess of LDA, with the carbamates **4** or **5**^[13, 14] to afford the corresponding adducts **6/7** in good yields^[15] and, in both cases, with diastereomeric ratios greater than 96:4 (Scheme 1). In general carbamates **5** provided the adducts **7** essentially as single diastereomers



Scheme 1. Asymmetric Mannich-type synthesis of β -amino acids. a) ref. [12]; b) LDA (3.5 equiv), THF, -78°C , 1 h then **4** or **5** (2 equiv), THF, -78°C , 15 min; c) H_2 (1 atm), Pd/C, EtOAc, RT, $(\text{Boc})_2\text{O}$; d) $(\text{NH}_4)_2\text{Ce}(\text{NO}_3)_6$ (3 equiv), $\text{CH}_3\text{CN}/\text{H}_2\text{O}$, 0°C , 16 h. Boc = *tert*-butoxycarbonyl, Cbz = benzyloxycarbonyl, LDA = lithium diisopropylamide, Tol = tolyl.

(Table 1), while the carbamates **4** led to slightly lower reaction diastereoselectivities, typically 98:2 for carbamates with linear side chains, and 96:4 for those bearing branched side chains. For stereochemical correlation purposes the adducts **6** were converted into the *N*-Boc derivatives **7** in a one-pot procedure.^[16] The assigned configuration for the adducts was established by a single-crystal X-ray structure analysis of the β -amino ketones **7a** and **7j**.^[17] The configuration for the other adducts was assigned by analogy.

The excellent diastereoselectivity attained with *N*-[1-aryl-sulfonyl]alkyl carbamates derived from either nonenolizable or enolizable aldehydes is of particular interest in that it provides, through oxidative cleavage of the acyloin moiety, β -alkyl- β -amino acids.^[18] For example, the β -amino acid **8a**, the *N*-Cbz β -homovaline **8d**, and the *N*-Boc- β -homophenylglycine **9j** were formed in yields of 81, 86, and 80%, respectively, by prolonged exposure of the adducts **6a**, **6d**, and **7j** to a threefold excess of ammonium cerium nitrate (CAN) in acetonitrile/water. Thus, β -amino acids appropriately protected at the amino function are obtained in an enantiomerically pure form,^[19] along with the recovery of the starting (1*R*)-(+)-camphor.^[20] This result is also particularly important because it makes it economically viable to use the more expensive

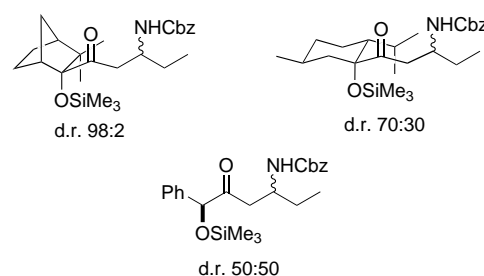
Table 1. Mannich-type synthesis of β -amino ketones **6** and **7**.^[a]

	R	R ¹	Product 6/7		
			d. r. ^[b]	yield[%] ^[c]	m. p. [$^{\circ}\text{C}$]
a	CH_3CH_2	Cbz	98:2	94	98–99
		Boc	> 98:2 ^[d]	71	120
b	$\text{CH}_3(\text{CH}_2)_2$	Cbz	98:2	66	82–84
		Boc	> 99:1	60	61–62
c	$\text{CH}_3(\text{CH}_2)_3$	Cbz	> 98:2 ^[d]	80	104–105
		Boc	98:2	82	87–88
d	$(\text{CH}_3)_2\text{CH}$	Cbz	98:2	60	77–78
		Boc	> 99:1	90	92–94
e	PhCH_2CH_2	Cbz	98:2	65	86–89
f	$(\text{CH}_3)_2\text{CHCH}_2$	Cbz	97:3	55	oil
g	$\text{BnOCH}_2\text{CH}_2$	Cbz	98:2	81	103–105
h	$\text{c-C}_6\text{H}_{11}$	Cbz	96:4	54	133–136
i	$\text{c-C}_6\text{H}_{11}\text{CH}_2$	Cbz	> 99:1	72	105–106
j	Ph	Boc	> 98:2 ^[d]	60	104–105

[a] Reactions carried out on a 4 mmol scale. [b] Diastereomeric ratios determined by HPLC analysis, using a LiChrospher 100 RP-18 column and mixtures of $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ as eluant ($T = 40^{\circ}\text{C}$). [c] Yields of **6** and **7** after purification of the crude product by column chromatography and/or crystallization from *n*-hexane. [d] Determined by ^{13}C NMR spectroscopy.

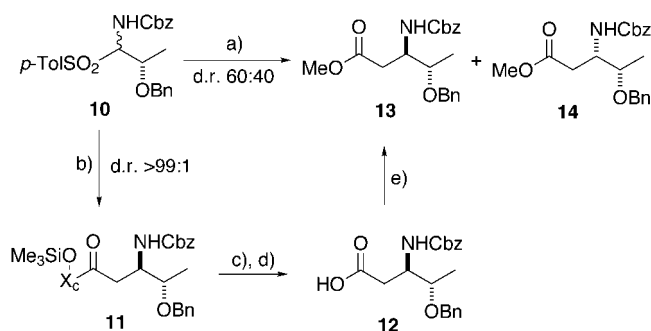
(1*S*)-(-)-camphor isomer, which would lead to the corresponding enantiomeric β -amino acids. Both (*R*)- and (*S*)- β -amino acids are found in natural products^[18b] and are also very interesting as potential building blocks for β -lactam antibiotics^[21] and for β -peptides.^[22]

To gain further insight into the importance of the present enolate model, three representative chiral α -oxymethyl ketones were also examined. Scheme 2 illustrates that of



Scheme 2. Mannich-type adducts from some selected α -silyloxymethyl ketones and carbamate **4a**. The configuration at the newly created stereogenic center was not determined. The diastereomeric ratios were determined by HPLC.

these ketones only that derived from (1*R*)-(-)-camphenilone was comparable in terms of efficiency to **3**. Therefore, owing to the advantageous preparation and low cost of the methyl ketone **3** it should be, from a practical standpoint, the reagent of choice for highly diastereoselective “acetate” aza-aldol reactions. In this respect, the example of double asymmetric induction^[23] depicted in Scheme 3 demonstrates the potential scope of **3**. Carbamate **10**, which shows essentially no diastereofacial selectivity against the lithium enolate of methyl acetate, reacted with the lithium enolate of **3** to produce **11**, essentially as the sole isomer, in 70% yield. This result was also consistently reproducible when mixtures of **10** with different epimeric compositions were employed.^[24] In addition, desilylation of **11** and subsequent oxidative cleavage of the acyloin moiety in the resulting intermediate afforded



Scheme 3. Diastereoselective synthesis of β -homo-*allo*-threonine **12**. a) CH₃CO₂Me, LDA (3.5 equiv), THF, -78 °C, 1 h then **10**, 15 min; b) **3**, LDA (3.5 equiv), THF, -78 °C, 1 h then **10**, 15 min; c) TBAF, THF, RT, 5 min; d) (NH₄)₂Ce(NO₃)₆ (3 equiv), CH₃CN-H₂O, 0 °C, 1 min; e) Me₃-SiCHN₂, MeOH/C₆H₆. TBAF = tetrabutylammonium fluoride.

the *N*-Cbz-*O*-benzyl- β -homo-*allo*-threonine **12** in 75 % yield. The diastereomeric purity of this compound was established by both ¹³C NMR and HPLC analyses of the corresponding methyl ester **13**, and comparison with the chromatograms from the mixture of **13/14**. The determination of isomeric ratios, the preparation procedures, and the analytical data for compounds **6a–j**, **7a–b**, **7j**, **8a**, **8d**, **9j**, **11**, and **12** can be found in the Supporting Information.

In summary, an asymmetric Mannich-type reaction that involves the use of acetylene and α -sulfonyl carbamates as the consumable organic materials is described for the first time.

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