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Stereoselective and N-terminal selective α-alkylation of peptides using a pyridoxal model compound as a chiral N-terminal activator

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Stereoselective and N-terminal selective α-alkylation of peptides is achieved using a pyridoxal model compound as an N-terminal activator which also functions as a chiral auxiliary.

Peptides containing unnatural amino acid(s) are gathering much attention because of their biochemical and medicinal properties. Although such peptides are generally synthesized by sequential coupling of the respective amido acids prepared independently,[1] several methods for the direct modification of peptides have also been reported.[2–4] Most of these methods, however, deal with the alkylation of peptides at sites other than the N-terminal position and seem to lack generality. In addition, their stereoselectivities depend on the stereochemistries of the peptides employed and are not always sufficient.[2,3] In contrast, direct N-terminal modification of peptides appears to be of great utility for the synthesis of peptides including unnatural amino acids, particularly where applied to combinatorial chemistry, as both liquid- and solid-phase peptide syntheses are generally achieved by sequential coupling from a C-terminal amino acid. O'Donnell and co-workers recently reported an interesting method utilizing this idea: direct N-terminal selective α-alkyltion of peptides *via* imine formation.[4] The only serious problem with their method is the lack of stereoselectivity. If the α-alkylation takes place with predictable stereoselectivity, this method would be more useful and versatile. In order for this method to be applicable to the synthesis of various peptides with predictable stereochemistry, it is desirable that the stereoselectivity of the α-alkylation is induced only by an external chiral auxiliary, and is not influenced by the neighbouring chiral centre on the peptide sequence. Here we report a useful method for N-terminal selective and stereoselective α-alkylation of peptides using a chiral pyridoxal model compound bearing an ionophore function.

Taking into account the application of the reaction not only to liquid-phase synthesis but also to solid-phase synthesis, we investigated the benzylation of the aldimines prepared from L-Ala-NHBn and some chiral pyridoxal models under various conditions.‡ As a consequence, we found that the reaction with pyridoxal model **1** having a chiral ansa-structure§ in the presence of $LiClO_4$[5] and DBU was the most effective for the present purpose and stereoselectively afforded the α-benzylated product (*R*)-**4** (X = NHBn) and recovered **1** (79%) after an acidic treatment (run 1 in Table 1 and Scheme 1).

These conditions were employed for the alkylation of peptides **2** (X = AA-OBn) and the results are summarized in Table 1.¶ The peptide-aldimine **3** (X = L-Ala-OBn) prepared from L-Ala-L-Ala-OBn and **1** was also stereoselectively benzylated at the N-terminal position without any detectable racemisation at the C-terminal α-position *via* these sequential reactions (run 2).‖ As expected, the stereochemistry at the N-terminal α-position was not related to the stereoselectivity at all (run 3). In order to examine the influence of the stereogenic centre at the neighbouring C-terminal α-position on the stereoselectivity of the alkylation, dipeptides L-Ala-Gly-OBn, L-Ala-L-Val-OBn, L-Ala-D-Ala-OBn, and L-Ala-D-Val-OBn were chosen as substrates. The reaction of peptides without a stereogenic centre or with a bulkier alkyl group at the C-terminal position similarly gave good *R*-stereoselectivity (runs 4 and 5). Moreover, in the reactions of the peptides having a D-amino acid at the C-terminal position, the same predominantly *R*-configuration was gained with slightly lower stereoselectivity (runs 2 *vs.* 6 and 5 *vs.* 7). It is noteworthy and quite significant that neither the stereochemistry nor the size of the

Table 1 Alkylation of aldimines **3** with RBr

	Substrate **2**						Product **4**	
Run	R^1	R^2	X	R	M^+	*t*/h	Yield (%)[a]	*R*:*S*[b]
1	Me	H	NHBn	Bn	Li	2.5	60	83:17
2	Me	H	L-Ala-OBn	Bn	Li	4	51	86:14
3	H	Me	L-Ala-OBn	Bn	Li	4	53	86:14
4	Me	H	Gly-OBn	Bn	Li	4	46	83:17
5	Me	H	L-Val-OBn	Bn	Li	4.5	50	88:12
6	Me	H	D-Ala-OBn	Bn	Li	4	49	74:26
7	Me	H	D-Val-OBn	Bn	Li	5	51	73:27
8	Me	H	L-Ala-OBn	4-$O_2NC_6H_4CH_2$	Li	4	50	85:15
9	Me	H	L-Ala-OBn	CH_2=$CHCH_2$	Li	4.5	48	73:27
10	Me	H	L-Ala-OBn	CH≡CCH_2	Li	4.5	56	84:16
11	Me	H	L-Ala-L-Ala-OBn	Bn	Li	5	48	86:14
12	Me	H	L-Ala-OBn	Bn	none	7	38	21:79
13	Me	H	L-Ala-OBn	Bn	Na	5	38	26:74
14	Me	H	L-Ala-OBn	Bn	K	7	32	23:77

[a] Isolated yield based on substrate **2**. [b] The stereochemistries of the products (*R*)- and (*S*)-**4** (X = L-Ala-OBn) were confirmed by comparing with an authentic sample (*S*)-**4** (X = L-Ala-OBn) prepared from L-Ala and (*S*)-(α-Bn) Ala-OBn, which had been obtained using our previous method (ref. 5). The stereochemistries of other products **4** were assigned as shown from their (*R*)-MTPA amides by comparing their ^1H and ^{19}F NMR data with those of the (*R*)-MTPA amide of authentic (*S*)-(α-Bn)Ala-L-Ala-OBn and (*S*)-(α-Bn)Ala-OBn. Excepting runs 1 and 4, the ratio was determined from the ^1H NMR spectra. In the runs 1 and 4, the ratios were determined from the ^1H and ^{19}F NMR spectra of the corresponding (*R*)-MTPA amides.

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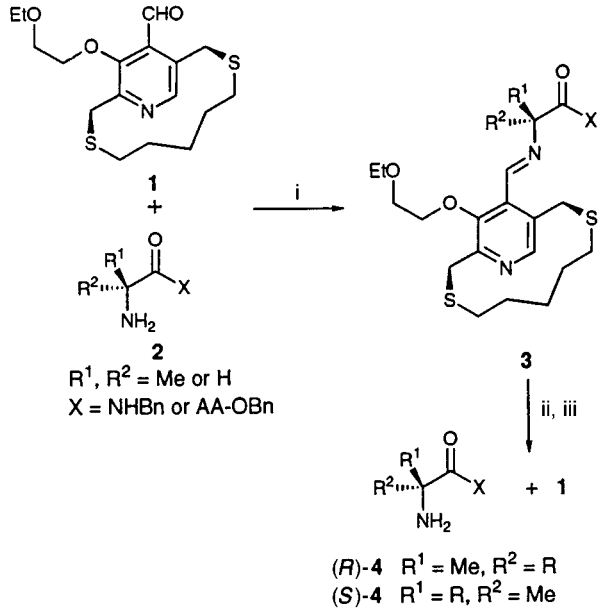

Scheme 1 *Reagents and conditions*: i, CH_2Cl_2, room temp.; ii, RBr, $MClO_4$, DBU, MeCN, 0 °C; iii, $TsOH·H_2O$, AcOEt, room temp., 30 min

substituent at the C-terminal α-position of the peptides affected the stereoselectivity. In addition, alkylation with other alkyl bromides proceeded successfully with similar stereoselectivities (runs 8–10). Tripeptide L-Ala-L-Ala-L-Ala-OBn was also stereoselectively benzylated under the same conditions (run 11). These findings show that compound **1** can work as an external chiral auxiliary as well as an N-terminal activator, at least in the synthesis of peptides with neutral amino acids at the neighbouring position.

Interestingly, the reaction without Li^+ or with other alkali metal ions was found to show the reverse stereoselectivity (runs 12–14). Concerning the reaction mechanism, ^1H NMR analysis of the peptide-aldimine **3** in the absence and presence of Li^+ revealed that the rotation of the C4–C4′ bond shown in Fig. 1 was induced only by the addition of Li^+.[5] The stereoselectivities obtained in the absence and presence of Li^+ appear to be attributable to these preferred conformations. Although the detailed mechanism has yet to be determined, predominant attack of the electrophile on the enolates from the side of the ansa-loop (*i.e.* upper side in Fig. 1) in the respective conformations could explain the stereoselectivities.**

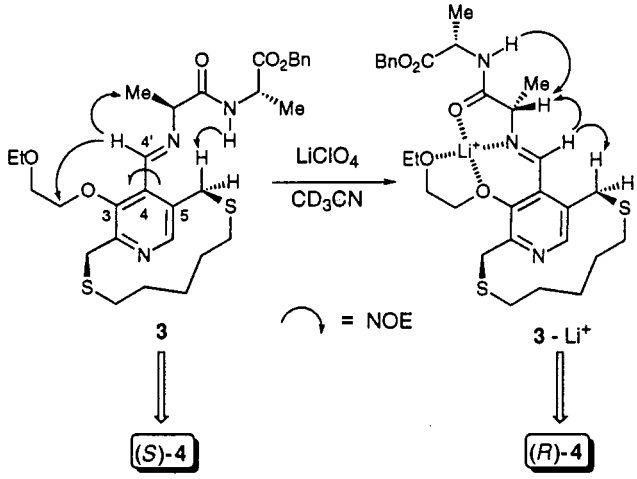

Fig. 1 Selected NOE data for **3** in the absence and presence of Li^+

In the present study, we have demonstrated the first example of stereoselective and N-terminal selective α-alkylation of peptides using a chiral pyridoxal model as an N-terminal activator which also functions as a chiral auxiliary. This α-alkylation reaction could be incorporated into standard sequential peptide syntheses, and could provide a novel method for the stereoselective synthesis of unnatural peptides, in particular, for construction of unnatural peptide libraries.††

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Notes and References

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‡ Although we first applied the pyridoxal model compound and the reaction conditions which had previously been effective for the asymmetric alkylation of α-amino esters (ref. 6) to this reaction, the desired stereoselectivity was not obtained. Hence, reactions with pyridoxal models having a chiral ionophore chain at C-3 and/or a chiral ansa-structure in the presence of various organic bases and metal ions were examined. Details will be reported in a full article.

§ The pyridoxal derivative **1** was synthesized from the 3-hydroxy derivative (ref. 7) according to the literature procedure (refs. 5, 6).

¶ *General procedure*: The peptide-aldimine was prepared according to the previously described procedure (ref. 5). To a stirred solution of peptide-aldimine **3** (0.10 mmol) and $LiClO_4$ (32.2 mg, 0.30 mmol) in MeCN (1 ml) was added DBU (29.9 μl, 0.20 mmol) at 0 °C. After stirring for 5 min at the same temperature, BnBr (13.2 μl, 0.11 mmol) was added and the mixture was stirred at 0 °C for the period indicated in Table 1. The reaction mixture was diluted with AcOEt (10 ml) and washed with cold water and cold brine. To the organic layer, $TsOH·H_2O$ (38.6 mg, 0.20 mmol) was added and the mixture was stirred for 30 min at room temperature and partitioned with Et_2O and water. The Et_2O phase was worked-up as usual and the residue was purified by SiO_2 column chromatography (AcOEt–hexane = 1 : 2) to give recovered **1** (70–80%). The aqueous phase was basified with $NaHCO_3$ and extracted with AcOEt. Usual work-up and purification with SiO_2 column chromatography (AcOEt) yielded the benzylated peptide **4**.

‖ This was confirmed by the fact that the (*R*)-MTPA amide derived from the benzylated dipeptide was shown to be a mixture of only two diastereomers based on the N-terminal α-position by ^1H and ^{19}F NMR analyses.

** The ansa-loop could push the other substituents out of the side of the ansa-loop and consequently make the other side crowded, which might allow the electrophile to approach from the same side of the ansa-loop. See also ref. 8.

†† Further extensions to the synthesis of longer peptides and to solid-phase synthesis are in progress.

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