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Asymmetric Access to Peptidyl β^3-Aldehydes by Coupling of *N*-Phthalyl α-Amino Acids with a Synthetic Heterocyclic β-Amino Aldehyde Precursor

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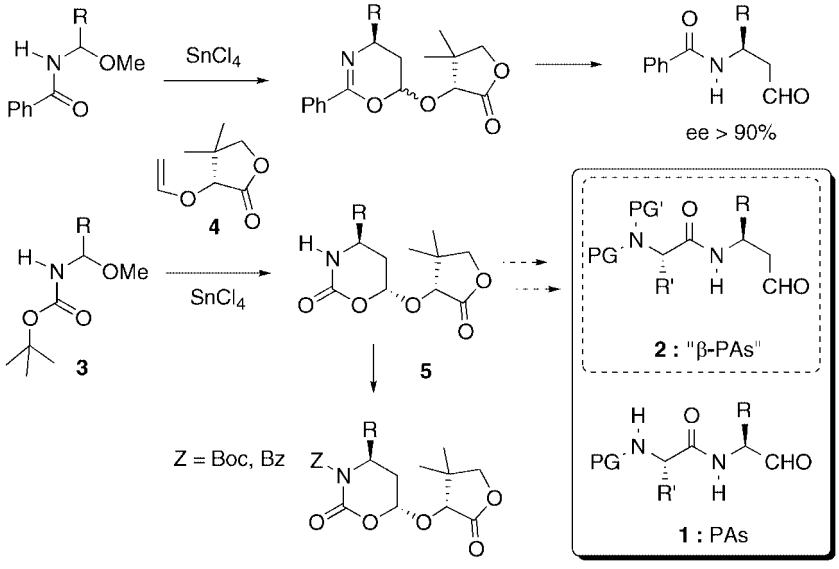
Asymmetric access to novel *N*-protected (di)peptidyl β^3-aldehydes ("β^3-PAs") has been achieved through direct coupling of a chiral non-racemic 6-alkoxytetrahydrooxazinone with *N*-phthalyl L-α-amino acids. Kinetic resolution allows for the fruitful use of racemic amino acids in this process. Acidic hydrolysis of the diastereomerically pure, coupling products leads to the title *N*-phthalyl-β^3-PAs in high yields.

Introduction

C-terminal peptide aldehydes (PAs) **1** have been the focus of considerable interest since their initial discovery as natural products.[1] They have been found to be inhibitors of different classes of proteolytic enzymes.[2] The inhibitory properties arise from the tetrahedral, hydrated C-terminus aldehyde function, which mimics the transition state of the substrate in the active site of the protease.[3] Further, PAs have been extensively used in peptide chemoselective ligation[4] and in pseudo-peptide chemistry, particularly for the synthesis of reduced peptides[5] or carbapeptides.[6] Methods for the preparation of peptide aldehydes that involve solution and solid-phase synthesis are well documented, most of which include the final release of the aldehyde group by reduction of the ester or Weinreb amide, by hydrolysis of the acetal, by oxidation of the alcohol, or by ozonolysis of the olefin.[4a,7] Little exploration has been conducted in the field of C-terminal β-peptide aldehydes[8] that show biological features of specific interest. The extensive evaluation and use of such β-derivatives require new synthetic methods that are able to provide substantial diversity in the design of the β-amino aldehyde subunits.



Scheme 1.

We have disclosed an enantioselective access to β-amido aldehydes through $SnCl_4$-promoted heterocycloaddition of

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(*R*)-*O*-vinyl pantolactone with *N*-benzoyl benzaldimine *N*,*O*-acetals and subsequent acid hydrolysis of the dihydrooxazines so obtained (Scheme 1).[9] More recently, we have demonstrated that under similar conditions the heterocyclic reaction of *N*-Boc derivatives **3** with (*R*)-*O*-vinyl pantolactone **4** led to the formation of new tetrahydrooxazinones **5** with a high facial selectivity (>98:2).[10] We envisioned that this novel class of heterocyclic compounds **5** could display a specific ability to undergo *N*-acylation with amino acids and to lead to the C-terminal β-peptide aldehydes **2** after ring-opening reaction.

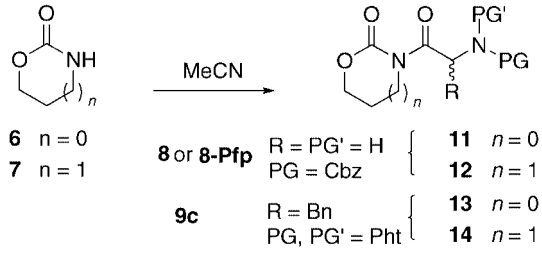
We were encouraged by preliminary results that demonstrated that *N*-Boc and *N*-Bz derivatives of **5** could be cleanly produced under standard conditions.[10] We report here the access by this pathway to β^3-dipeptide aldehydes **2** ("β^3-PAs") through direct coupling of adducts **5a** (R = Ph) with *N*,*N*-diprotected α-amino acids (PG, PG′ = Phthalyl).

Results and Discussion

Only a few reports described the coupling reaction of amino acids with five-membered cyclic carbamates[11] and, to the best of our knowledge, no example involving six-membered carbamates have been reported. The peptide-like coupling of adducts **5** was particularly challenging since it required the finding of activating conditions that could offset the low nucleophilicity of **5** with respect to its hemiacylal functionality and to the stereogenicity of the amino acid moiety. In initial experiments, we observed that methods based on prior *N*-metalation were inefficient – treatment of **5a** with LiHMDS or NaH led to decomposition even at low temperature. Thus, in a preliminary study involving model carbamates **6**–**7**, we considered some *N*-acylation procedures that avoided the use of strong bases (Table 1).

Tomasini and co-workers described the *N*-acylation of oxazolidinones with pentafluorophenyl amino esters by using diisopropylethylamine (DIEA) and 4-dimethylaminopyridine (DMAP) in DMF.[11] Applying this method to Cbz-Gly-OPfp (**8-Pfp**) proved to be efficient with 2-oxazolidinone **6**, but gave no results with tetrahydrooxazinone **7** (entries 1, 2). The direct model coupling reaction was then tested with *N*-protected amino acids [**8** and (±)-**9c**] by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl) as the coupling reagent (see Figure 1). With 2-oxazolidinone **6**, the combination of EDCI·HCl with one equivalent of DMAP and excess *N*-methylmorpholine (NMM) afforded the desired product **11** in good yield

Table 1. Coupling reactions of **6** and **7** with amino acids.



Entry	Starting material	Amino acid	Coupling conditions [equiv.]	Product	Yield [%]
1	**6**	**8-Pfp**	DMAP (1), DIEA (4)	**11**	63
2	**7**	**8-Pfp**	DMAP (1), DIEA (4)	**12**	0
3	**6**	**8**	EDCI·HCl (1.5), DMAP (1), NMM (4)	**11**	68
4	**7**	**8**	EDCI·HCl (1.5), DMAP (1), NMM (4)	**12**	21
5	**6**	(±)-**9c**	EDCI·HCl (1.5), DMAP (1), NMM (4)	**13**	70
6	**7**	(±)-**9c**	EDCI·HCl (1.5), DMAP (1), NMM (4)	**14**	65

(entry 3) from **8**. Unfortunately, when this method was applied to the tetrahydrooxazinone **7**, it led to the coupling product **12** in low yield (entry 4), along with many other by-products. In contrast, under the same conditions with *N*-phthalyl amino acid (±)-**9c** coupled with **6** and **7** gave rise to the products **13** and **14**, respectively, in similar acceptable yields (entries 5, 6). Therefore, assuming that the low yield observed for **12** was a consequence of the closed nucleophilicity of both NH groups on **8** and **7**, we concluded that *N*,*N*-diprotection of the amino acid was presumably necessary for the coupling reaction of tetrahydrooxazinones such as **5a**. Further experiments demonstrated that the presence of DMAP was also essential for the coupling: the use of any other reagent without DMAP, such as EDCI·HCl/DIPEA, EDCI·HCl/pyridine, EDCI·HCl/NMM, EDCI·HCl/NMM/*N*-hydroxy succinimide (HOSu), or EDCI·HCl/NMM/BOP, was unsuccessful for peptide formation.

The use of a phthalimido protecting group has the advantage of being easily introduced and removed. However,

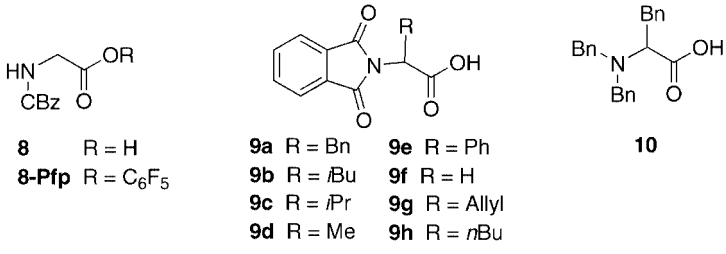
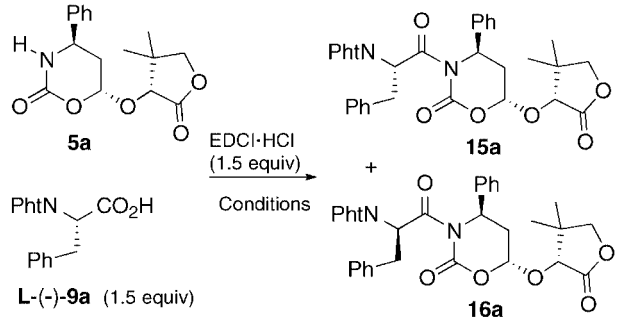


Figure 1. Amino acids and derivatives used in this study.

the use of the EDCI·HCl/DMAP/NMM system for coupling chiral *N*-phthalyl amino acids is not recommended because of the high propensity of the corresponding acyl (4-dimethylamino)pyridinium salt to undergo racemization.[12] This failure was evidenced in the reaction of L-(–)-**9a** with adduct **5a** when the same EDCI·HCl/DMAP/NMM system (Table 2, entry 1) was used.[13] At room temperature, formation of both isomers **15a** and **16a** in a 4:1 ratio was thus observed in 51% total yield. At a lower temperature (0 °C, entry 2), the coupling reaction did not take place at all. At a higher temperature (40 °C, entry 3), only degradation took place. Longer reaction times at room temperature (entry 4) did not improve the yield and diminished the diastereoselectivity to a large extent. The amount of DMAP used for this reaction was also crucial, since when it was reduced to 0.5 equiv., no coupling product was formed (entry 5). To our delight, using 1.1 equiv. of DMAP without NMM (entry 6) led to the desired coupling product in 54% yield, with a fairly pure diastereomeric form (*dr* = 98:2); a further small increase in the amount of DMAP dramatically influenced the diastereomeric ratio (*dr* = 65:35 with 1.3 equiv., entry 7). No coupling product was formed when the amount of DMAP was decreased to 0.9 equiv. (entry 8), which suggests that a stoichiometric amount of DMAP as base is prerequisite for coupling. Temperature is also an important factor, since at 30 °C extensive formation of epimer **16a** occurred (*dr* = 73:27, entry 9).

Table 2. Coupling study between L-(–)-**9a**[a] and **5a**.

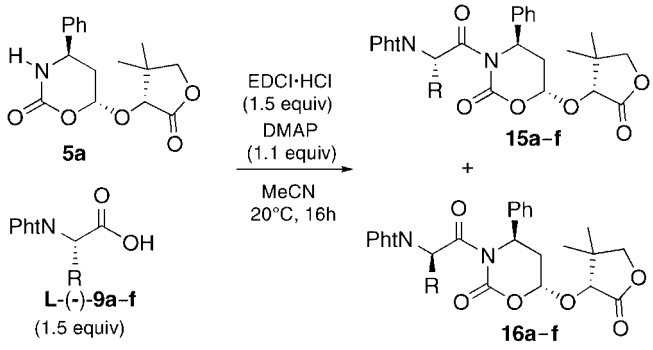


Entry	DMAP [equiv.]	NMM [equiv.]	Time [h]	*T* [°C]	Yield [%][b]	**15a/16a**[c]
1	1	4	7	20	51	80:20
2	1	4	7	0	0[d]	–
3	1	4	7	40	0[e]	–
4	1	4	48	20	54	64:36
5	0.5	4	16	25	0[d]	–
6	1.1	0	16	20	51	98:2
7	1.3	0	16	20	55	65:35
8	0.9	0	16	20	0[d]	–
9	1.1	0	16	30	57	73:27

[a] See Figure 1. [b] Isolated mixture of diastereomers. [c] Determined by 400 MHz ^{1}H NMR spectroscopy. [d] Starting material **5** was recovered. [e] Degradation products.

The use of EDCI·HCl (1.5 equiv.) and DMAP (1.1 equiv.) at 20 °C in CH_3CN allowed the efficient coupling reaction between *N*-phthalyl amino acid L-()-**9a** and **5a** without substantial formation of the epimer.[14] The scope of the coupling reaction was then explored with various *N*-phthalyl amino acids (Table 3). All L-amino acids tested with **5** provided the coupling products in moderate to good yields and with a high diastereopurity (≥97:3). To our surprise, almost no coupling product was isolated when the substrate was D-(+)-**9a** or D-(+)-**9c**. These results show a chiral discrimination in the coupling reaction of **5a**, which suggests that a kinetic resolution might be expected when starting from racemic *N*-phthalyl amino acids.

Table 3. Coupling reaction of amino acids with **5a**.



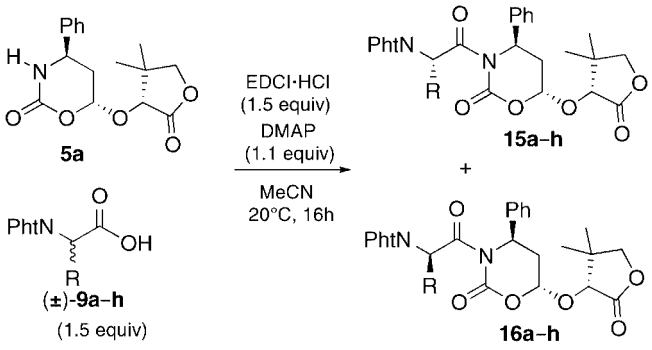
Entry	Amino acid	R	Major product	Yield[a] [%]	**15/16**[b]
1	L-(–)-**9a**	Bn	**15a**	51	98:2
2	L-(–)-**9b**	*i*Bu	**15b**	64	97:3
3	L-(–)-**9c**	*i*Pr	**15c**	67	100:0
4	L-(–)-**9d**	Me	**15d**	60	98:2
5	**9f**	H	**15f**	43	–
6	D-(+)-**9a**	Bn	**16a/15a**	Trace	–
7	D-(+)-**9c**	*i*Pr	**16c/15c**	Trace	–

[a] Isolated yield. [b] Determined by 400 MHz ^{1}H NMR spectroscopy.

The resolution of racemic acid chlorides or mixed anhydrides by using lithiated chiral oxazolidinones is well documented,[15] but no paper to date has reported the resolution of racemic amino acids.[16] We envisioned to obtain diastereomerically enriched coupling products **15** starting from racemic amino acids, through kinetic resolution, by direct coupling with **5a**. Under the same conditions as above,[17] we found that the expected coupling products **15** could be obtained from racemic amino acids with good to high diastereoselectivities in moderate to good yields (Table 4). The failure of (±)-**9e** (entry 5) to couple might result because it is easily deprotonated by DMAP to form an enolate and thus lacks electrophilicity. The selectivities strongly depended on the size of R^1: *N*-phthalylphenylalanine and *N*-phthalylvaline led to high selectivity, while *N*-phthalylalanine gave rise to a relatively low selectivity. Except for **15h** and **16h** (R = *n*Bu, entry 7), diastereoisomers **15** and **16** of the *N*-phthalyl-protected derivatives were easily separated by flash chromatography with THF/*n*-hexane as eluent. The absolute configuration (4*R*,6*R*,2′*R*) of the diastereoisomer **16g** was established unambiguously by its X-ray structure

(Figure 2), and those of each pair of diastereomers **15a–h/16a–h** was thus determined by ^{1}H NMR spectroscopic comparisons with **15g** and **16g.**

Table 4. Coupling reactions of (±)-**9a–h** with **5a**.



Entry	Amino acid	R	Major product	Yield **15**[a] [%]	Yield **16**[a] [%]	**15/16**[b]
1	(±)-**9a**	Bn	**15a**	48 (62)[c]	<2	97:3
2	(±)-**9b**	*i*Bu	**15b**	47 (63)[c]	10	83:17
3	(±)-**9c**	*i*Pr	**15c**	55 (73)[c]	0	100:0
4	(±)-**9d**	Me	**15d**	44 (59)[c]	20	69:31
5	(±)-**9e**	Ph	–	0		
6	(±)-**9g**	Allyl	**15g**	47 (64)[c]	17	74:26
7	(±)-**9h**	*n*Bu	**15h**	64[d] (80)[c]	–[e]	94:6[e]

[a] Isolated yield of pure diastereomer, unless noted. [b] Based on isolated diastereomer ratio, unless noted. [c] Isolated yield of **15/** 0.75 equiv. of L-(–)-**9**. [d] Isolated yield of mixture **15/16**. [e] Based on 400 MHz ^{1}H NMR spectroscopy on mixture.

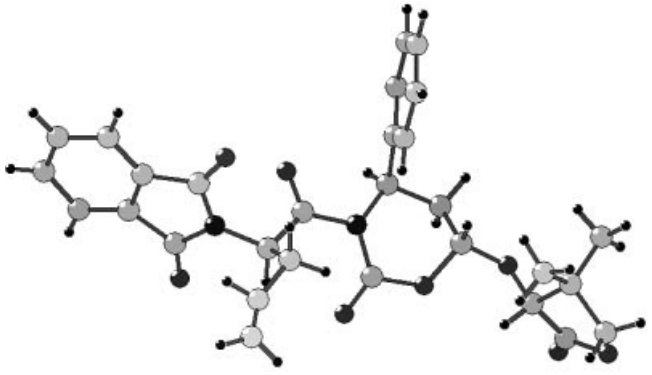
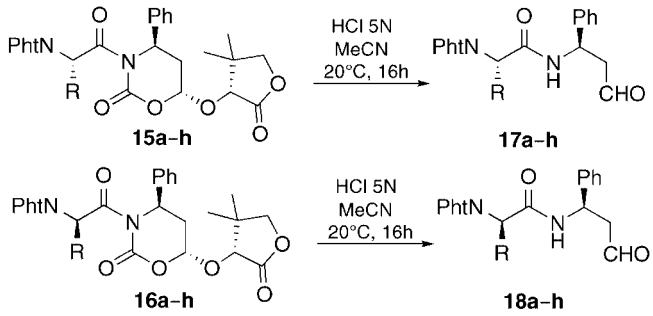


Figure 2. X-ray crystal structure of **16g**.

Indeed, the differences specifically observed between the ^{1}H NMR spectroscopic data of the heterocyclic protons suggest that the *trans* tetrahydrooxazinone rings adopt two different conformations in **15** and **16**. According to the crystalline structure of **16g**, both diastereomers **16** are thus characterized by a conformation in which the *N,O*-acetal proton 6-H appears antiperiplanar to the *trans* vicinal 5-H proton (dd, δ = 5.31–5.38 ppm, J = 8–9 and 3.6–4 Hz). In contrast, for all diastereomers **15** such a conformation cannot be assumed since neither the 6-H proton (dd, δ = 5.35–5.39 ppm, J = 5.6–6.8 and 3.6–5.6 Hz) nor the 4-H proton (pseudo triplet, δ = 5.56–5.68 ppm, J = 5.2–6.8 Hz) seems to exhibit an antiperiplanar position with a vicinal 5-H proton. As another significant difference, the carbinol proton of the pantolactone ring is always appear more downfield for "*L*"-isomers **15** (δ = 4.30–4.35 ppm) than for "*D*"-isomers **16** (δ = 4.01–4.11 ppm).

With diastereomerically pure coupling products in hand, our next aim was to carry out the ring-opening reaction to form β-peptide aldehydes. Previous results showed that on treatment with 6 N HCl in THF, the *N*-benzoyl derivative of **5a** could be converted into the corresponding *N*-benzoyl β-amino aldehyde without loss of the stereogenicity albeit in low yield.[10] Fortunately, we found that coupling products **15** and **16** were efficiently converted into the corresponding aldehydes after simple treatment with 5 N HCl in CH_3CN overnight at room temperature in excellent yields. After simple extraction with ethyl acetate and washing with H_2O to remove (–)-pantolactone, the desired aldehydes were obtained in high purity without further chromatographic purification (Table 5).

Table 5. Formation of *N*-Pht-β-peptide aldehydes **15/16**.



Entry	Coupling product	R	Aldehyde	Yield [%]
1	**15a**	Bn	**17a**	91
2	**16a**	Bn	**18a**	87
3	**15b**	*i*Bu	**17b**	92
4	**16b**	*i*Bu	**18b**	86
5	**15c**	*i*Pr	**17c**	89
6	**15d**	Me	**17d**	93
7	**16d**	Me	**18d**	87
8	**15f**	H	**17f**	88
9	**15g**	Allyl	**17g**	84
10	**16g**	Allyl	**18g**	95

Conclusion

In summary, in this work we report on an efficient method for the asymmetric synthesis of unprecedented (*L,L*) β-peptide aldehydes, which involved the direct coupling reaction of tetrahydrooxazinone **5a** with *N*-phthalyl L-amino acids by using EDCI·HCl and DMAP, and the subsequent acid hydrolysis of the coupling products. An efficient kinetic resolution is observed when racemic *N*-phthalyl amino acids are used. Another main synthetic advantage of the method is the easy diastereomeric separation of coupling products **15** and **16**. These interesting features were observed only in the *N*-phthalyl series, in contrast with the negative results obtained with *N*-benzoyl or *N,N*-dibenzyl amino acids. The scope of the coupling reaction, studies on the mechanism of the kinetic resolution, access to *N*-

monoprotected β-peptide aldehydes, and extensions to solid-phase parallel synthesis are under way in our laboratory.

Supporting Information: (See footnote on the first page of the article.) Experimental procedures, spectroscopic and other data for the new compounds, the X-ray structure determination procedure, and the crystal data for **16g** are included.

Acknowledgments

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[13] (*trans*)-(4*R*,6*R*)-Tetrahydrooxazinone **5a** used for the present study contained 4–8% of other diastereoisomers. All coupling products isolated (**15–16**, Table 2–Table 4) were found to exhibit the same (4*R*,6*R*) ring configuration ("*exo* β"[10]).

[14] By applying the same conditions to the *N*,*N*-dibenzyl analogue (L)-(–)-**10** of (L)-(–)-**9a** led to disappointing stereochemical results (**15i/16i** 83:17).

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[17] Unexpectedly, attempts to increase the yield of **15a** by using 2 equiv. of (±)-**9a** failed, no matter what the amount of EDCI·HCl used (1.5–2 equiv.).

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