COMMUNICATION

Efficient stereoselective synthesis of peptidomimetics containing hydroxyethylamine dipeptide isosteres utilizing the aza-Payne rearrangement and *O, N*-acyl transfer reactions

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A novel methodology utilizing the aza-Payne rearrangement and *O, N*-intramolecular acyl transfer reactions for the synthesis of peptidomimetics containing hydroxyethylamine dipeptide isosteres (HDIs) is described. This methodology is useful for the stereoselective synthesis of HDI-containing pseudopeptides, and applicable to combinatorial chemistry using solid-phase techniques.

Introduction

Use of hydroxyethylamine dipeptide isosteres (HDIs) has been well documented as backbone replacements of amide bonds in the position P1–P1′ of aspartyl protease inhibitors. Recently, the great success of HIV protease inhibitors containing HDIs has caused a remarkable increase in general interest in these isosteres.[1] We wish to use HDI-containing peptidomimetics for the synthesis of inhibitors against β-secretase,[2] an example of an aspartic protease as a HIV protease. β-Secretase, which is a therapeutic target for Alzheimer's disease, cleaves the amyloid-β precursor protein to generate the *N*-terminal region of the amyloid-β protein. Herein, we report a useful methodology utilizing the aza-Payne rearrangement[3] and *O, N*-intramolecular acyl transfer reactions[4] for the solid-phase synthesis of HDI-containing peptidomimetics.

Results and discussion

Initially, a model peptidomimetic **10** was prepared in the solution phase as shown in Scheme 1. Starting from D-serine, an *N*-2,4,6-trimethylphenylsulfonyl (Mts)-protected amino alcohol **2a** was readily synthesized by the addition of Pr^{i}MgCl to a protected D-serinal, which was prepared by the DIBAL reduction of **1**. Subsequent aziridinyl ring formation using the Mitsunobu reaction[5] afforded an *N*-Mts-protected (and activated) aziridine **3a**. The Grignard reaction of D-serinal exclusively afforded the *threo*-amino alcohol **2b** (**2a** : **2b** = 1 : > 99), whereas the reagent in the presence of $ZnCl_2$ and LiCl preferentially afforded the *erythro*-amino alcohol **2a** (**2a** : **2b** = 3 : 1). Stereochemical assignments for the diastereomers **2a** and **2b** were confirmed by conversion to the acetonide derivatives, followed by NOE experiments.[6] After removal of the TBS group of **3a**, the aza-Payne rearrangement of the resulting aziridinylmethanol with NaH in THF gave the requisite *N*-Mts-protected epoxy amine **4a** stereo- and regio-selectively, as previously reported.[3] Treatment of epoxy amine **4a** with an aspartic acid derivative **5** in Pr^{i}OH–DIPEA (*N*,*N*-diisopropylethylamine) afforded an HDI **6**, *via* the regioselective S_N2 ring-opening reaction at position 3 of **4a**, as previously reported.[7] Mts-amino protection is critical both for the Mitsunobu reaction and the aza-Payne rearrangement. However, it is difficult to remove the Mts group while retaining the Bu^t group. Thus, elongation of the peptide chain from the secondary hydroxy group of **6** is judged to be a useful alternative. After the Boc protection of the imino group of **6**, esterification of the secondary hydroxy group of **7** with Fmoc-Asn(Trt)-OH afforded a protected *O*-acyl compound **8**. Removal of all the protecting groups of **8** except for the Fmoc group by treatment with 1 M TMSBr–thioanisole in TFA, resulted in the *O*-acyl compound **9**. Compound **9** was incubated in phosphate buffer at pH 7.3 containing CH_3CN at 0 °C for 1 h to yield an *N*-acyl compound **10** in 66% yield *via* the *O, N*-intramolecular acyl migration .[4] The progress of this migration was monitored by reversed-phase HPLC. The retention time of the *O*-acyl compound **9** was shorter than that of the *N*-acyl compound **10** in the HPLC solvent system with H_2O and CH_3CN, both containing 0.1% (v/v) TFA. As a result, the present procedure has been proven to be a useful methodology for the stereoselective synthesis of HDI-containing pseudopeptides using solution-phase techniques.

Next, we attempted to apply this method to the solid-phase synthesis of a substrate-based β-secretase inhibitor **17**. A dipeptidyl resin **11** was constructed using standard fluoren-9-ylmethyloxycarbonyl (Fmoc)-based solid-phase techniques on a Rink-Amide-CLEAR resin[8] (Peptide Institute, Inc., Osaka, Japan) (Scheme 2). The CLEAR resin particles swell in a wide range of solvents including H_2O, CH_2Cl_2, DMF or CH_3OH. Treatment of epoxy amine **4b** with **11** in Pr^{i}OH–DIPEA afforded an HDI-containing peptidyl resin **12**. Since Pr^{i}OH is a critical solvent for the epoxy ring-opening reaction, the CLEAR resin is a suitable choice for the solid-phase reactions in terms of the degree of swelling in Pr^{i}OH. The Boc protection of the imino group of **12** and the subsequent esterification of the secondary hydroxy group with Fmoc-Asn(Trt)-OH gave a protected *O*-acyl compound **13**.[9] After deprotection of the Fmoc group of **13**, Fmoc–Val–OH was condensed on the α-amino group of the Asn(Trt) residue to yield the corresponding compound **14**. The removal of all the protecting groups of **14**, except for the Fmoc group, and the cleavage from the resin by treatment with the 1 M TMSBr–thioanisole system resulted in the *O*-acyl compound **15**. Subsequent incubation in phosphate buffer at pH 7.3 containing CH_3CN at 0 °C for 1 h yielded the corresponding *N*-acyl compound **16** *via* the *O, N*-intramolecular acyl transfer. The progress of this migration was monitored by reversed-phase HPLC. Deprotection of the Fmoc group yielded the desired HDI-containing compound **17** in 9.4% overall yield based on the Rink-Amide-CLEAR resin. The compounds bound to the resins, **12** and **13**, were characterized by mass spectra of the corresponding resin-free peptidomimetics that were cleaved from the resin and deprotected with the 1 M TMSBr–thioanisole system. Compound **17** was identical with the authentic sample, which was synthesized by the conventional solution-phase method. Furthermore, five pseudopeptides **18**–**22** were similarly synthesized in order to evaluate the applicability of the present method to combinatorial chemistry (Table 1). In all cases, the objective compounds with high purity were easily prepared in moderate overall yields.

In conclusion, the present procedure utilizing the aza-Payne rearrangement and the *O, N*-intramolecular acyl transfer reactions provides a convenient methodology for the preparation of HDI-containing peptidomimetics linked to a solid-phase. The

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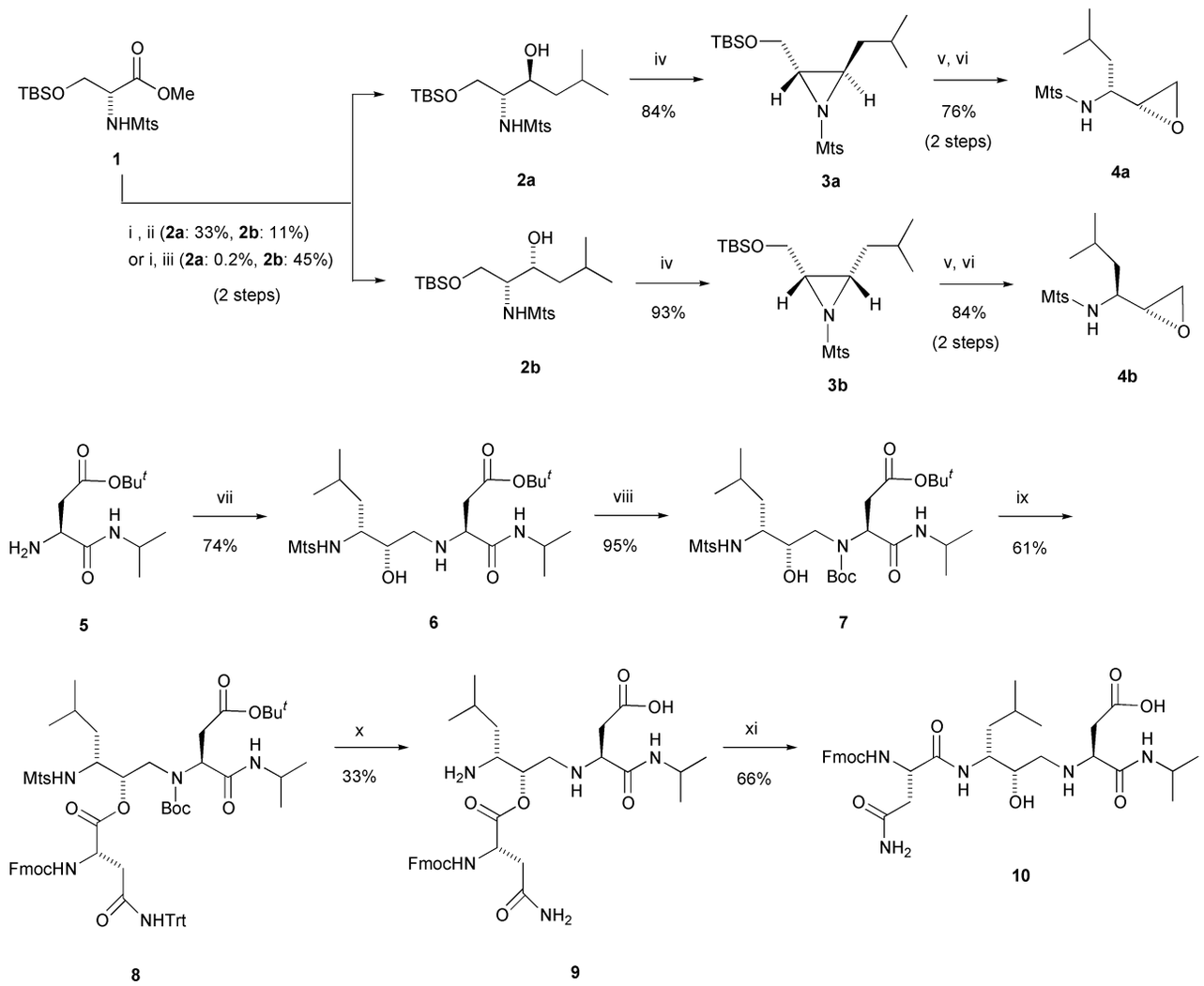
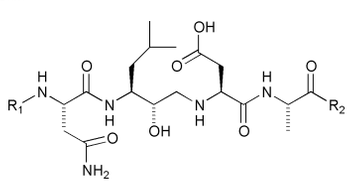


Scheme 1 Mts = 2,4,6-trimethylphenylsulfonyl, TBS = *tert*-butyldimethylsilyl, Fmoc = fluoren-9-ylmethyloxycarbonyl; *Reagents*: i, DIBAL; ii, Pr^{i}MgCl, $ZnCl_2$, LiCl; iii, Pr^{i}MgCl; iv, Ph_3P, DEAD in THF; v, 1 M Bu_4NF in THF; vi, NaH in THF; vii, **4a**, DIPEA, Pr*i*OH; viii, $(Boc)_2O$, Et_3N; ix, Fmoc-Asn(Trt)-OH, DCC, DMAP; x, 1 M TMSBr–thioanisole–TFA, *m*-cresol, ethane-1,2-dithiol (EDT); xi, phosphate buffer pH 7.3, CH_3CN.

Table 1 Structures of synthetic pseudopeptides containing HDI and their overall yields



Compound number	R^1	R^2	Yield (%)
18	H–	$–NH_2$	14.3
17	H–Val–	$–NH_2$	9.4
19	H–	–Glu–NH_2	10.2
20	H–Val–	–Glu–NH_2	7.5
21	H–	–Glu–Phe–NH_2	13.8
22	H–Val–	–Glu–Phe–NH_2	6.9

procedure has been proven to be applicable to combinatorial chemistry.

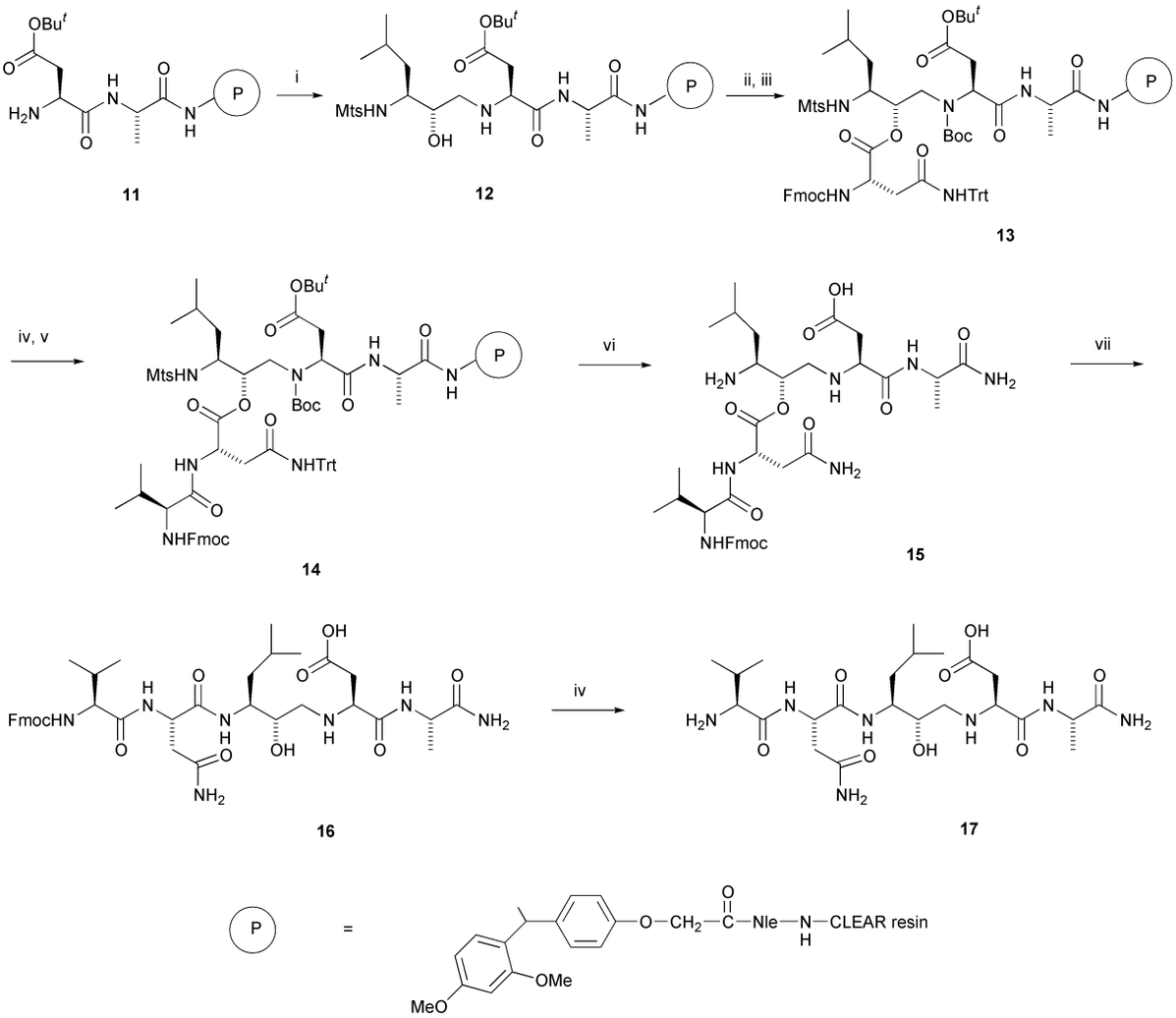
Experimental

Compound 10

Compound **9** (10.4 mg, 11.8 μmol) was dissolved in phosphate buffer (pH 7.3, 13 mL) and CH_3CN (2 mL) at 0 °C. After 1 h incubation, an aliquot (10 μL) was sampled and examined by analytical HPLC (33% CH_3CN isocratic mode in the solvent system using H_2O and CH_3CN, both containing 0.1% (v/v) TFA, the retention time of compound **9** = 16.4 min; the retention time of compound **10** = 24.4 min). The complete conversion from compound **9** to **10** was confirmed by the HPLC profile of the 1 h incubation. The crude compound **10** in solution was purified by preparative HPLC and lyophilized to give a white powder of compound **10**: yield 5.9 mg (66.4% based on compound **9**). $[a]_D^{24}$ −6.87 (*c* 0.291 in DMSO); δ_H(600 MHz; DMSO-d_6) 8.9–8.3 (br, 1H), 8.23 (m, 1H), 7.89 (d, *J* 7.4, 2H), 7.77 (d, *J* 7.6, 1H), 7.71 (t, *J* 8.2, 2H), 7.42 (t, *J* 7.3, 2H), 7.42 (m, 1H), 7.34 (d, *J* 7.6, 1H), 7.33 (d, *J* 7.3, 2H), 7.20–6.94 (m, 2H), 5.52 (br s, 1H), 4.33 (m, 1H), 4.26 (m, 1H), 4.21 (m, 2H), 3.93 (m, 1H), 3.83 (m, 1H), 3.70 (m, 1H), 3.62 (m, 1H), 3.14–2.96 (m, 2H), 2.88–2.68 (m, 2H), 2.43 (m, 1H), 1.57–1.50 (m, 1H), 1.40–1.34 (m, 2H), 1.05 (d, *J* 6.6, 3H), 1.01 (d, *J* 6.4, 3H), 0.83 (d, *J* 6.6, 3H), 0.76 (d, *J* 6.4, 3H); IS-MS Found: $(M + H)^+$, 640.5 ($C_{33}H_{45}N_5O_8$ requires *M* + H, 640.3346); FAB-MS Found: $(M + H)^+$, 640.3367 (diastereoselection >99 : 1 from NMR analysis. A diastereomer, derived from epimerization in the esterification of **7**, was not detected.)

Compound 17

The protected pseudopeptidyl resin **14** (49.0 μmol) was treated with 1 M TMSBr–thioanisole in TFA (20 mL) in the presence of *m*-cresol (800 μL) and EDT (400 μL) at 0 °C for 2 h. After removal of the resin by filtration, the filtrate was concentrated *in vacuo*, followed by the addition of Et_2O to precipitate the product. After washing with Et_2O, the product **15** was dissolved in phosphate buffer (pH 7.3, 10 mL) and CH_3CN (2 mL) at 0 °C



Scheme 2 *Reagents*: i, **4b**, DIPEA, Pr^{i}OH; ii, $(Boc)_2O$, Et_3N; iii, Fmoc-Asn(Trt)-OH, DCC (1,3-diisopropylcarbodiimide), DMAP; iv, 20% (v/v) piperidine–DMF; v, Fmoc–Val–OH, DCC, HOBt (*N*-hydroxybenzotriazole); vi, 1 M TMSBr–thioanisole–TFA, *m*-cresol, EDT; vii, phosphate buffer pH 7.3, CH_3CN.

to perform the *O, N*-acyl transfer reaction in the same way as in the synthesis of compound **10**. After concentration of H_2O and CH_3CN, the crude product **16** was treated with 20% (v/v) piperidine–DMF at rt for 15 min, followed by evaporation. The product was purified by preparative HPLC and lyophilized to give a white powder of compound **17**: yield 3.6 mg (overall 9.4% based on the Rink-Amide-CLEAR resin). $[\alpha]_D^{25}$ − 32.7 (*c* 0.153 in H_2O); δ_H(600 MHz; DMSO-d_6) 9.1–8.3 (br, 1H), 8.63 (d, *J* 7.0, 1H), 8.58 (d, *J* 7.0, 1H), 8.03 (br s, 2H), 7.82 (d, *J* 8.3, 1H), 7.41 (s, 1H), 7.37 (s, 1H), 7.11 (s, 1H), 6.98 (s, 1H), 5.56 (br s, 1H), 4.57–4.52 (m, 1H), 4.25 (m, 1H), 4.09 (m, 1H), 3.69–3.60 (m, 3H), 3.07 (m, 1H), 2.90 (m, 2H), 2.68 (m, 1H), 2.49 (dd, *J* 15.8 and 6.0, 1H), 2.45 (dd, *J* 15.8 and 7.3, 1H), 2.09–2.02 (m, 1H), 1.59–1.52 (m, 1H), 1.36 (m, 2H), 1.25 (d, *J* 7.1, 3H), 0.94 (d, *J* 6.9, 3H), 0.92 (d, *J* 6.9, 3H), 0.86 (d, *J* 6.6, 3H), 0.77 (d, *J* 6.5, 3H); IS-MS Found: $(M + H)^+$, 546.0 ($C_{23}H_{43}N_7O_8$ requires *M* + H, 546.3251); FAB-MS Found: $(M + H)^+$, 546.3236.

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9 HPLC analysis of the deprotected–cleaved peptide from **13** revealed that 11–13% epimerization occurred at this esterification step: DMAP 0.1 eq., 12%; 0.2 eq., 13%; 0.3 eq., 11%. The side product derived from this epimerization was eliminated by preparative HPLC at the final step (the purification of **17**). NMR analysis of purified **17** did not indicate a diastereomer (diastereoselection >99 : 1).