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Facile Synthesis of α,α-Diisobutylglycine and Anchoring Its Derivatives onto PAL-PEG-PS Resin

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Abstract: α,α-Diisobutylglycine has been synthesized using a Pd-mediated dialkylation of ethyl nitroacetate as a key first step. The free ααAA is N^{α}-protected and has been applied to the assembly of conformationally constrained peptide analogues. Mixed anhydrides from BOP-Cl and Fmoc-ααAA-OH are used for anchoring ααAAs onto a trialkoxybenzyl linker on PEG-PS grafted support, upon which a *β*-strand mimic with difficult sequence is assembled in a superior quality.

There is an increased interest in the synthesis and use of symmetrically α,α-disubstituted amino acids (ααAAs) in controlling peptide secondary structures.[1,2] Recent approaches to ααAAs via alkylation of α-nitroacetate[3] and Ni−Schiff base complex[4] allow practical preparation of a variety of ααAAs in good chemical yields. However, highly hindered ααAAs that possess branched side chains, such as α,α-diisobutylglycine **3a** (Dibg),[5] have not been readily synthesized.[6,7] Though Dibg was traditionally prepared via the Bucherer−Bergs method,[8] there were major flaws associated with this approach due to the high resistance to the formation and hydrolysis of the hydantoin, which in turn gave a very low overall yield of the desired product. In our effort to assemble a number of *β*-strand mimics containing Dibg and other ααAAs, we needed to develop a practical method for preparing a large quantity of protected Dibg that is suitable for solid-phase peptide synthesis. Herein we report an efficient synthesis of Dibg via Pd-mediated diallylation of α-nitroacetate. Additionally, some of the peptides we are currently making require a hindered ααAA at the C-terminus. Such peptides are difficult to assemble as coupling of the ααAA to the solid support generally affords low yields.[9] Thus, we also report here an improved method for anchoring ααAAs onto PAL-PEG-PS resin via mixed anhydrides.

We have found α-nitroacetate a very useful synthon for the preparation of ααAAs with and without side chain functionality, where the α,α-dialkylated nitroacetate was produced as a key precursor to the target compound.[3] Since direct dialkylation of nitroacetate with branched alkyl halides proved to be nonproductive (Table 1, entry 1), we tried activated allyl halides for the alkylation under different conditions. While allyl iodide gave the diallylated acetate in a moderate yield (Table 1, entry 5), the branched allyl iodide, 3-iodo-2-methylpropene only gave a low yield of the diallylated nitroacetate (Table 1, entry 3), and the major product of this reaction was a mixture of mono-C-allylated and O-allylated derivates. In both cases, allyl bromides only gave trace amount of desired compounds (Table 1, entries 2 and 4).

In search for an efficient method for dialkylation of α-nitroacetate with branched allyl halides or it equivalents, we thought that use of "activated" allylic groups as the electrophiles would be a feasible approach. Allylic activation with palladium templates has long been used for alkylation of preformed carbanions.[10] The previously reported Pd-catalyzed allylic alkylation of α-substituted nitro carboxylic acids gave quarternary nitro esters in good yields.[11,12] Muzart's group and Lopez's group reported Pd-mediated alkylation of allyl acetate by nitro

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(5) Abbrevations: Aib, α-aminoisobutyric acid; Api, 4-aminopiperidine-4-carboxylic acid; BOP-Cl, bis(2-oxo-3-oxazolidinyl)phosphinic chloride; Dbzg, α,α-dibenzylglycine; DCE, 1,2-dichloroethane; Dibg, α,α-diisobutylglycine; DIEA, *N*,*N*-diisopropylethylamine; Dpg, α,α-dipropylglycine; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOAt, hydroxy-7-azabenzotriazole; MALDI-MS, matrix-assisted laser desorption ionization mass spectrometry; PAL, 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid; PEG-PS, poly(ethylene glycol)polystyrene graft; PyAOP, 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluoro-phosphate; SPPS, solid-phase peptide synthesis; TPS, triisopropylsilane; Z, benzyloxycarbonyl.

(6) While alkylations of the Ni−Schiff base complex with unbranched alkyl halides lead to the formation of ααAAs in high yields, this method has not been extended for the synthesis of branched ααAAs. The alkylation with branched alkyl halides gave predominately mono-alkylated products under attempted conditions. Ellis, T. K.; Martin, C. H.; Tsai, G. M.; Ueki, H.; Soloshonok, V. A. *J. Org. Chem.* **2003**, 68, 6208−6214.

(7) Synthetic approaches to Dibg were attempted via condensation of symmetric ketones with $PhCH_2NH_2$ or Hofmann degradation of substituted cyanoacetic ester; however, both approaches gave a very low overall yield of the desired product. See: (a) Freitas, A. M.; Maia, H. L. S. *Peptide, Proc. Eur. Pept. Symp.* 1989, 13−15. (b) Lin, K.-H.; Li, L.; Huang, Y.-T. *Science & Technol. China* **1948**, *1*, 5−10.

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TABLE 1. Alkylation of Ethyl Nitroacetate with Allyl Halides and Allyl Acetates

entry	electrophile	method[a]	reagent	*T* (°C)	prod no.	yield[b] (%)
1	$(CH_3)_2CHCH_2I$	A	Bu_4NI	50	**1c**	trace[c]
2	$CH_2{=}C(CH_3)CH_2Br$	A	Bu_4NBr	60	**1a**	trace[c]
3	$CH_2{=}C(CH_3)CH_2I$	A	Bu_4NI	60	**1a**	<10
4	$CH_2{=}CHCH_2Br$	A	Bu_4NBr	60	**1b**	trace[d]
5	$CH_2{=}CHCH_2I$	A	Bu_4NI	60	**1b**	45[d]
6	$CH_2{=}C(CH_3)CH_2OAc$	B	$Pd(PPh_3)_4$	50	**1a**	92 (90)[e]
7	$CH_2{=}C(CH_3)CH_2OAc$	B	$Pd(PPh_3)_4$	25	**1a**	80
8	$CH_2{=}CHCH_2OAc$	B	$Pd(PPh_3)_4$	50	**1b**	93

[a] Method A: solvent, DMF; 1.5 M nitroacetate, 0.15 M Bu_4NI (or Bu_4NBr when an alkyl bromide was the electrophile); 2.1 equiv of DIEA, 2.1 equiv of electrophile. Method B: solvent, THF; 0.6 M nitroacetate, 5 mol % Pd catalyst, 2.1 equiv of electrophile, 2.1 equiv of DIEA. [b] All percentage yields refer to isolated products. [c] Major products were mono-C-alkylated and O-alkylated derivatives. [d] Reaction results were previously reported in ref 3. [e] The number in parentheses represents the reaction yield when 2 mol % of Pd catalyst was added. Unless mentioned, 5 mol % Pd catalyst was used for the activation.

esters and other nucleophiles, where both mono- and diallylated products were usually obtained.[13] Tsuji et al. reported decarboxylation−allylation of allyl methyl carbonate using Pd as a catalyst where the diallylated nitroacetate was produced in a good chemical yield.[14] Pd-catalyzed cross-coupling reactions of nitro esters with branched allyl acetate in an aqueous medium has also been performed in which the diallylated acetate was formed as a minor product in a low yield.[15]

The synthetic approach to α,α-diisobutylglycine proposed herein employes Pd(0)-mediated diallylation of ethyl α-nitroacetate with 2-methylallyl acetate in the presence of a tertiary amine (Scheme 1). In our experiments, ethyl α-nitroacetate was treated with 2 equiv of DIEA to form nitroacetate anion,[3] which subsequently attacked the Pd(II)−allyl complex to give diallylated nitroacetates in excellent yields (Table 1, entries 6−8). $Pd(Ph_3P)_4$ (5 mol %) was used for the formation of Pd(II)−allyl complex in most attempts, and in one attempt only 2 mol % of the Pd(0) catalyst was used for the activation where a high yield of the desired product was also obtained (Table 1, entry 6). It was observed that the alkylation could be improved by heating, especially for the reaction with branched allyl acetate, where a 92% yield was obtained at 50 °C in comparison to a 80% yield at room temperature (Table 1, entries 6 and 7). There was no O-alkylated nitroacetate isolated under attempted conditions. The Pd catalyst was easily removed by the addition of polymer-supported PPh_3 resin, and the diallylated acetate **1** was obtained in an excellent yield.

The highly efficient dialkylation of the ethyl nitroacetate occurred through a Pd-aided "net S_N2 displacement". One of the reasons that the Pd-catalyzed allylation is so efficient relative to a direct S_N2 (or S_N2') reaction, even in sterically hindered cases, is that the transition state for C−C bond formation involves exo attack on a π-allyl−Pd complex rather than formation of a trigonal−bipyramidal intermediate that must occur in an S_N2 reaction. Trost demonstrated this difference in reactivity by a competition reaction of malonate anions with a substrate that had both primary bromide and an allylic acetate in the presence of Pd.[10] Alkylation of the malonate only occurred through the allylic acetate with the bromide left unchanged. In contrast, alkylation of the nitroacetate with alkyl halides (Table 1, entries 1−3) mainly goes through a regular S_N2 process.

To make the desired free ααAA, both the nitro group and the side chain alkene in **1a** needed to be reduced. On the basis of our previous work,[3] we found that the tertiary NO_2 group in nitro esters could be easily reduced to the free amine under Raney nickel-catalyzed hydrogenation. Thus, the reduction of the NO_2 and C=C group in **1a** was carried out in one step under hydrogen (50 psi) over T-1 Raney nickel to give the amino ester **2a** in a high yield (Scheme 1). This amino ester **2a** was then hydrolyzed in refluxing aq KOH−EtOH, followed by isolation through a cation-exchange resin column to give the free Dibg in a 91% yield. The free ααAA needed to be N^{α}-protected to allow for either solid-phase or solution-phase peptide synthesis. The N^{α}-protection of the amine group in **3a** was carried out using an in situ silylation procedure[16] where Fmoc-Cl, $(Boc)_2O$, or Z-Cl was added to the silylated ααAA in CH_2Cl_2 to provide Fmoc-Dibg-OH, Boc-Dibg-OH, and Z-Dibg-OH in very good chemical yields.

The main application for N^{α}-protected ααAAs in our ongoing projects is for assembly of conformationally constrained peptide mimics. In several designed peptides, an ααAA was at the C-terminus and thus needed to be directly attached to the solid support for C → N assembly of the peptide. Attachment of the C-terminal ααAA with a resin linker is typically difficult. For example, coupling of Fmoc-Dibg-OH onto PAL-PEG-PS resin (with NH_2 as the reactive functional group) using PyAOP, HATU, or amino acid fluorides, some of the conventional activation methods for difficult couplings,[17,18] only afforded moderate yields (Table 2, entries 1−3).

Similar results were obtained with Dpg couplings. Anchoring of the first amino acid residue to the solid support is one of the most critical steps in peptide synthesis, and the inefficient attachment significantly reduces the overall yield and also creates a deletion sequence that could complicate subsequent peptide purification.

BOP-Cl-mediated coupling has been utilized for assembly of peptide analogues with difficult sequences, such as peptides containing N-substituted amino acids.[18,19] We have also observed that preformed mixed anhydrides from BOP-Cl and Fmoc-L-amino acids could

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SCHEME 1

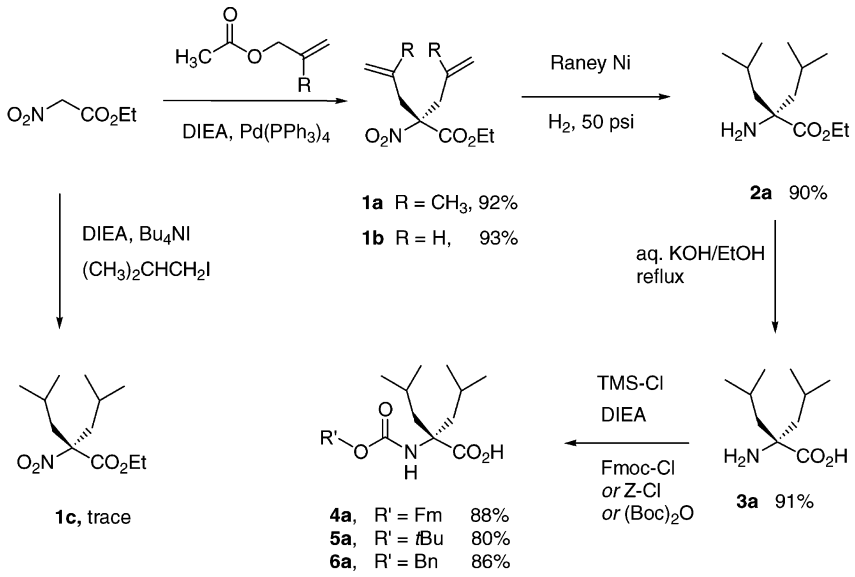
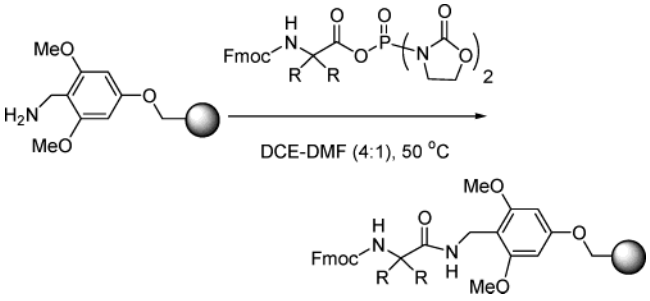


TABLE 2. Attachment of (A) Fmoc-Dibg-OH and (B) Fmoc-Dpg-OH with PAL-PEG-PS Resin

			coupling yield[b] (%)	
entry	coupling method[a]	base	A	B
1	PyAOP	DIEA	33 (25)[c]	46 (29)[c]
2	HATU	DIEA	41 (26)[c]	64
3	Fmoc-ααAA-F	DIEA	43	55
4	BOP-Cl[d]	none[e]	82	91

[a] All couplings were performed in DCE−DMF (4:1) for 8 h. Unless mentioned, all couplings were carried at 50 °C. [b] The coupling yield was determined by UV analysis of the Fmoc-deprotection. [c] The number in parentheses represents the coupling yield at 25 °C. [d] Preformed mixed anhydride was used in the coupling. The mixed anhydride was prepared by treatment of 1 equiv of Fmoc-ααAA-OH with 1 equiv of BOP-Cl with the presence of 1 equiv of DIEA in CH_2Cl_2 at 0 °C for 2 h. The CH_2Cl_2 was removed in vacuo, and the concentrated anhydride was taken up in DCE−DMF (4:1) for coupling. [e] DIEA was used in the formation of the mixed anhydride; no additional base was used in the coupling.

SCHEME 2



efficiently acylate the N-terminus of sterically hindered ααAAs.[17] Here we report our preliminary study on coupling ααAAs onto a solid support using mixed anhydrides. It was found that preformed mixed anhydrides from BOP-Cl and Fmoc-ααAA-OH residues could readily acylate the NH_2 group on PAL-PEG-PS resin (Scheme 2; Table 2, entry 4). There was no additional base added to the coupling, and the PS resin was suspended in the relatively nonpolar DCE−DMF (4:1) solvent at 50 °C to help the resin swell. On the basis of the excellent coupling

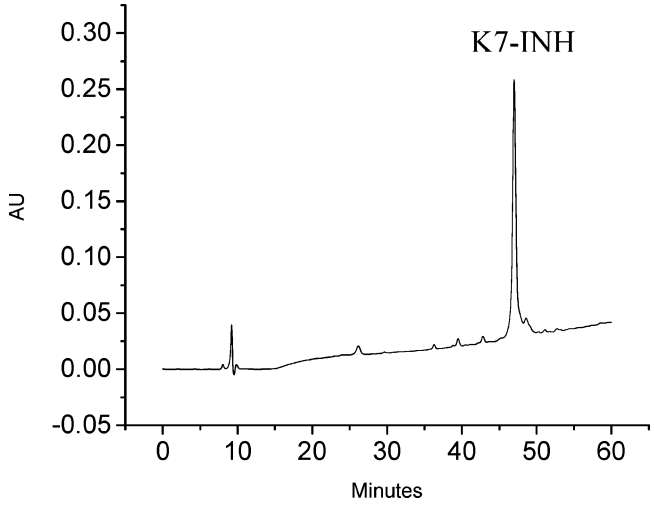


FIGURE 1. HPLC profile of crude peptide K7-INH: H-$(Lys)_7$-Dibg-Val-Dbzg-Phe-Dpg-NH_2. Column: Delta-Pak C_{18}, 15 μm, 300 Å, 8 × 100 mm. Eluent: (A) H_2O and 0.1% TFA; (B) MeCN and 0.1% TFA. Gradient: 10−70% B over 60 min. Flow rate: 1 mL/min.

result from the first amino acid attachment, we synthesized a peptide K7-INH containing three different ααAAs, H-$(Lys)_7$-Dibg-Val-Dbzg-Phe-Dpg-NH_2, using our previously reported symmetrical anhydride coupling protocol[17] and PyAOP/HATU activation methods. The highly efficient assembly of the difficult peptide was verified by HPLC (Figure 1) and by MALDI-MS. Currently, this peptide and other synthesized β-strand mimics are being investigated for their inhibitory activities against β-amyloid assembly, and the results will be reported in due course.

We have reported a practical synthesis of α,α-diisobutylglycine via Pd-mediated diallylation of α-nitroacetate. This method may be extended for the synthesis of a variety of sterically hindered symmetrical and asymmetrical ααAAs. Also, we have reported an improved method for coupling ααAAs to PAL-linker on PEG-PS resin using preformed mixed anhydrides. This coupling

method may be useful for anchoring other sterically demanding amino acids to solid supports. Based on our previous results and the findings described herein, we believe that BOP-Cl-mediated coupling may be generally used for the synthesis of conformationally constrained peptides having ααAAs at different sequence positions.

Experimental Section

Ethyl 2,2-Bis(2-methylallyl)-2-nitroacetate (1a). To a solution of ethyl nitroacetate (10.0 g, 75.1 mmol) in dry THF (110 mL) were added 2-methylallyl acetate (18.0 g, 157.1 mmol) and $Pd(PPh_3)_4$ (9.0 g, 7.8 mmol). After 15 min DIEA (20.1 g, 157.1 mmol) was added, and the reaction was stirred under argon for 8 h at 50 °C. The resulting solution was filtered over Celite while washing with THF (2 × 120 mL). The filtrate was concentrated, dissolved in 75 mL of EtOAc and washed with 10% K_2CO_3 (50 mL). To the organic layer was added 12 g of PS-PPh_3 (1.3 mmol/g, Argonaut) resin and the mixture was shaken for 25 min. The resin was filtered off and the filtrate was purified via column chromatography (EtOAc−Et_2O, 2:1) to provide a light yellow oil:[15] yield 16.5 g (92%); ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.96−4.94 (m, 2H), 4.81−4.79 (m, 2H), 4.24 (q, J = 7.2 Hz, 2H), 3.08−2.95 (m, 4H), 1.72-1.71 (m, 6H), 1.28 (t, J = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.6, 138.4, 117.2.3, 95.1, 62.7, 41.8, 23.2, 13.7. Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.81. Found: C, 59.42; H, 7.69; N, 5.56.

An Example of Synthesis of N^{α}-Protected Dibg. N^{α}-(9-Fluorenylmethoxycarbonyl)-2,2-diisobutylglycine (4a). TMSCl (8.8 g, 81.0 mmol) was added to a suspension of amino acid **3a** (7.4 g, 39.6 mmol) in dry CH_2Cl_2 (80 mL) and refluxed under N_2 for 8 h. The mixture was cooled to 0 °C, and DIEA (10.5 g, 81.0 mmol) and Fmoc-Cl (10.0 g, 38.6 mmol) were added. The reaction was allowed to warm to 25 °C and stirred for 20 h. The resulting mixture was concentrated in vacuo to provide a yellow solid which was then dissolved in EtOAc (80 mL). To this mixture, water was added (40 mL), and the solution was acidified to pH 2.0 with 2 N HCl. The separated organic layer was dried ($MgSO_4$) and concentrated in vacuo to provide a light yellow solid. The crude product was triturated in hexanes to afford a white solid (13.7 g, 88% yield): mp 132−133 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 7.90−7.28 (m, 8H), 6.48 (s, 1H), 4.33 (d, J = 6.7 Hz, 2H), 4.20 (t, J = 6.7 Hz, 1H), 2.03−1.97 (m, 2H), 1.63−1.48 (m, 4H), 0.80−0.76 (m, 12 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.7, 153.4, 143.7, 140.7, 127.6, 127.0, 125.0, 120.1, 64.9, 61.5, 46.7, 43.6, 23.8, 23.7, 23.1. Anal. Calcd for $C_{25}H_{31}NO_4$: C, 73.32; H, 7.63; N, 3.42. Found: C, 73.50; H, 7.76; N 3.52.

Solid-Phase Peptide Synthesis. The peptide H-$(Lys)_7$-Dibg-Val-Dbzg-Phe-Dpg-NH_2 was prepared using Fmoc methodology with Fmoc-PAL-PEG-PS resin (0.17 mmol, 0.16 mmol/g loading) as a solid support. Except for the last six Lys residues that were incorporated into the sequence via the PyAOP protocol (Fmoc-Lys(Boc)-OH, 4 equiv; PyAOP, 4 equiv; DIEA, 8 equiv; solvent DMF; 2 h for each coupling at 50 °C) on a Perseptive Biosystem Peptide Synthesizer, all amino acids were incorporated into the sequence manually. Dpg was attached to the solid support using the preformed mixed anhydride (8 equiv, 0.3 M) in DCE−DMF (4:1) at 50 °C. Capping of the resin was carried out by treating the resin with excess 0.2 M of Ac_2O in 0.28 M of DIEA (16 equiv) in DMF for 2 h. Phe and Dbzg were incorporated into the sequence using PyAOP with DIEA in DCE−DMF (1:1); Val residue was coupled to N-terminus of Dbzg via amino acid symmetrical anhydride method.[17] Dibg was coupled to Val using HATU (4 equiv), HOAt (4 equiv), DIEA (8 equiv), and Fmoc-Dibg-OH (4 equiv, 0.3 M) in DCE−DMF (4:1) at 50 °C. Lys residue was coupled to Dibg via symmetrical anhydride at 50 °C. Removal of Fmoc protection group before each coupling step was performed using DBU−piperidine−DMF (2:5:93) at 50 °C for 15 min. After each coupling step, coupling efficiency was examined by UV analysis of the Fmoc-deprotection (∼8 mg of resin in 1 mL of deblocking solution for 10 min). After the assembly, the resin was treated with a cleavage solution of TFA−TPS−phenol-H_2O (88:2:5:5, v/v/v) at room temperature for 2 h. After filtration, the filtrate was concentrated and added to cold ether and the precipitate was isolated by centrifugation. Peptide purification was performed by reversed-phase HPLC on a C_{18} column to yield 188 mg of crude product (81% crude purity) which was identified by MALDI-MS, m/z calcd $[M + H]^+$ 1710.4, obsd 1710.7. Semipreparative HPLC gave an overall 43% yield of pure peptide (> 99% purity).

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Supporting Information Available: Experimental details for **1b**, **2a**, **3a**, **5a**, **6a**; ^{1}H and ^{13}C NMR spectra for **1a**, **2a**, **3a**, **4a**, **5a**, and **6a**; methods for anchoring Dpg and Dibg onto the resin; MALDI mass spectrum of peptide K7-INH. This material is available free of charge via the Internet at http://pubs.acs.org.

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