Synthesis of a Vicinal Tricarbonyl Amide Derivative of L-Phenylalanine

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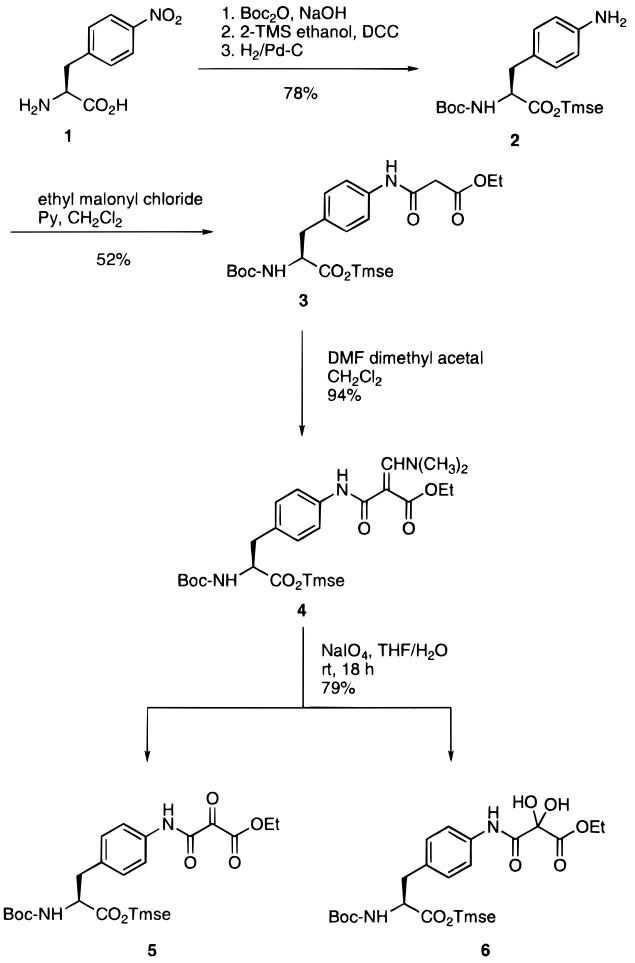
Unnatural amino acids have found wide utility for the modification of the physical properties and biological activities of peptides, peptide mimetics, and, more recently, proteins.[1] Due to their expanding utility, synthetic routes to novel unnatural amino acids, particularly those with unusual chemical properties, broaden the scope of what can be done in this area. Wasserman and co-workers have made significant contributions to the synthesis, structural characterization, and synthetic utility of the highly electrophilic vicinal 1,2,3-tricarbonyl moiety.[2] They have also utilized its reactivity to develop inhibitors of serine proteases by replacing the terminal carboxyl group in peptides with the vicinal tricarbonyl moiety so that the nucleophilic serine in the active site could form a covalent bond with the central carbonyl carbon.[3] We expect that the reactivity of the vicinal 1,2,3-tricarbonyl moiety might also find utility when incorporated into the side chains of amino acids. The resulting unnatural amino acids could be incorporated into proteins[1] and peptides to modify their physical and biological properties as well as to introduce a new reactive functionality which could form a covalent bond intramolecularly (e.g. with a serine OH reacting at the central carbonyl carbon) or intermolecularly (e.g. with a nucleophile present in the active site of an enzyme to which the modified peptide or protein binds). For this purpose, we have developed the first synthesis of the novel protected L-phenylalanine analog **5** containing a vicinal tricarbonyl amide moiety at the *para* position of the phenyl ring as shown in Scheme 1. In the course of developing this synthesis we also extended the scope of a new oxidation procedure to enamines such as **4** thereby providing more convenient access to vicinal tricarbonyl compounds via enamine precursors.

Commercially available *p*-nitro-L-phenylalanine hydrochloride **1** was protected as the *N*-Boc, 2-(trimethylsilyl)ethyl (Tmse) ester[4] under the indicated standard conditions. Subsequent hydrogenation of the *p*-nitro group gave **2** in 78% overall yield. Acylation of **2** with ethyl malonyl chloride gave **3** in 52% yield after flash chromatography. Conversion of malonate **3** to the enamine derivative **4** was accomplished in 94% yield using *N*,*N*-dimethylformamide dimethylacetal following the procedure of Wasserman and Han.[5] Our initial attempts to cleave oxidatively the enamine double bond in **4** to obtain **5** with the singlet oxygen or ozone methods used by Wasserman and Han[5] resulted in no reaction or only a 25% yield, respectively. Consequently, we were attracted to the recent report of Vetelino and Coe[6]

Scheme 1



wherein they achieved the oxidative cleavage of aryl enamines to aryl aldehydes with sodium periodate in aqueous THF at room temperature without added osmium tetroxide. We investigated the applicability of this method to the synthesis of vicinal 1,2,3-tricarbonyl functionalities using our present substrate **4** as an example. The difference between our substrate and the series of substrates investigated by Vetelino and Coe is that the enamine functionality to be oxidized in **4** is embedded immediately between two preexisting carbonyl groups whereas those previously explored were flanked only by an aromatic ring and therefore resulted in aromatic aldehyde products. Reaction of **4** with 3 equiv of $NaIO_4$ in aqueous THF for 18 h at room temperature resulted in a 79% combined yield of the vicinal tricarbonyl product **5** and the corresponding hydrate **6** after purification and separation (obtained in a ca. 1/1 ratio) by flash chromatography over silica gel. The isolation of the hydrate **6** was expected since the electrophilic vicinal 1,2,3-tricarbonyl moiety is known to hydrate readily.[2]

The vicinal tricarbonyl product **5** and its hydrate **6** could be readily distinguished by the ^{13}C NMR chemical shifts of the central carbon atom of the vicinal tricarbonyl moiety. For the nonhydrated tricarbonyl carbon in **5** the chemical shift was 178 ppm whereas after hydration to **6** the chemical shift moved upfield to 92 ppm in agreement with earlier ^{13}C NMR characterizations of other vicinal tricarbonyl compounds and their hydrates.[7]

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Experimental Section

All reagents were purchased from Aldrich Chemical Co., Milwaukee, WI, including *p*-nitro-L-phenylalanine monohydrate (**1**). All reactions were stirred under an atmosphere of dry N_2 and at rt unless noted otherwise. Solutions were concentrated *in vacuo* on a rotary evaporator with a water bath temperature of 30 °C unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed with precoated silica gel glass plates (0.25 mm plates Type GF, Analtech, Newark, DE). Visualization of TLC slides was by either iodine, UV, or ninhydrin (for **2**). Flash chromatography was carried out with silica gel 60 (230−400 mesh) from E. Merck. ^{1}H and ^{13}C NMR spectra are reported in ppm (δ) downfield from TMS. Elemental analyses were performed by Atlantic Microchemical Lab, Inc. Norcross, GA.

***N*-α-Boc-*β*-(*p*-Aminophenyl)-L-alanine 2-(Trimethylsilyl)-ethyl Ester (2).** To a solution of *p*-nitro-L-phenylalanine monohydrate (**1**) (5.34 g, 23.4 mmol) in a mixture of dioxane (50 mL), water (25 mL), and 1 N NaOH (25 mL) precooled in an ice−water bath was added di-*tert*-butyl dicarbonate (7.55 g, 34.6 mmol). The reaction was allowed to warm to rt and then continued for 3 h longer. The reaction mixture was concentrated to about 30 mL, cooled in an ice−water bath, covered with a layer of EtOAc (80 mL), and acidified with a 1 N $KHSO_4$ to pH 2−3. The aqueous phase was extracted with EtOAc (3 × 350 mL), and then the extracts were combined, washed with water (350 mL), dried over $MgSO_4$, and concentrated. The residue was crystallized from EtOAc/hexane to give 6.98 g (96%) of *N*-Boc-*p*-nitro-L-phenylalanine: TLC, R_f = 0.77 (3/1 EtOAc/MeOH); ^{1}H NMR (DMSO-d_6) δ 1.29 (s, 9H), 2.90−3.00 (m, 1H), 3.15−3.20 (m, 1H), 4.17 (m, 1H), 7.23 (d, J = 8.5 Hz, 1H), 7.53 (d, J = 8.6 Hz, 2H), 8.16 (d, J = 8.6 Hz, 2H), 12.74 (s, 1H).

To a solution of *N*-Boc-*p*-nitro-L-phenylalanine (3.10 g, 10.0 mmol) in acetonitrile (10 mL) were added pyridine (7.2 mL) and 2-(trimethylsilyl)ethanol (1.7 mL, 12 mmol). After the reaction mixture had been cooled in an ice/water bath, 1,3-dicyclohexylcarbodiimide (2.3 g, 11 mmol) was added, and the mixture was stirred in the ice/water bath for 1 h and then kept in the refrigerator overnight. A solution of oxalic acid (189 mg) in DMF (0.4 mL) was added. About 0.5 h later, the precipitated dicyclohexylurea was removed by filtration and washed with EtOAc. The combined filtrate and washings were extracted with 1 N HCl (3 × 350 mL) followed by 10% aqueous $NaHCO_3$ (350 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography using increasing concentrations of EtOAc in hexane as the eluent to give 4.0 g (97%) of *N*-Boc-*p*-nitro-L-phenylalanine 2-(trimethylsilyl)ethyl ester: TLC, R_f = 0.76 (1/2 EtOAc/hexane); ^{1}H NMR (DMSO-d_6) δ 0.0 (s, 9H), 0.80−0.90 (m, 2H), 1.30 (s, 9H), 2.90−3.05 (m, 1H), 3.05−3.15 (m, 1H), 4.10 (t, J = 8.3 Hz, 2H), 4.15−4.25 (m, 1H), 7.35 (d, J = 8.5 Hz, 1H), 7.51 (d, J = 8.6 Hz, 2H), 8.13 (d, J = 8.6 Hz, 2H); ^{13}C NMR ($CDCl_3$) −1.0, 18.0, 28.8, 39.0, 54.8, 64.8, 80.7, 124.1, 130.9, 144.8, 147.7, 155.5, 171.8.

To a solution of 7.8 g (19 mmol) of *N*-Boc-*p*-nitro-L-phenylalanine 2-(trimethylsilyl)ethyl ester in methanol (20 mL) was added 10% Pd−C (810 mg), and then the mixture was hydrogenated on a Parr apparatus at 50 psi H_2 and rt. The reaction was complete within 3 h as determined by TLC. The catalyst was removed by filtration through Celite, and the pad was rinsed with additional methanol. The combined filtrate was concentrated to give 6.6 g (84%) of **2**: TLC, R_f = 0.35 (1/2 EtOAc/hexane); ^{1}H NMR (DMSO-d_6) δ 0.0 (s, 9H), 0.80−0.90 (m, 2H), 1.31 (s, 9H), 2.59−2.75 (m, 2H), 3.90−4.00 (m, 1H), 4.06 (t, J = 7.8 Hz, 2H), 4.86 (s, 2H), 6.42 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.1 Hz, 2H), 7.07 (d, J = 7.8 Hz, 1H); **2** tends to air oxidize readily and was therefore used the same day for the preparation of **3**.

***N*-α-Boc-*β*-[[*p*-(Ethoxymalonyl)amino]phenyl]-L-alanine 2-(Trimethylsilyl)ethyl Ester (3).** To a solution of **2** (6.55 g, 17.0 mmol) and pyridine (57 mmol, 4.6 mL) in dry CH_2Cl_2 (20 mL) cooled to 0 °C under an Ar atmosphere was added dropwise a solution of ethyl malonyl chloride 3.0 mL (23 mmol) in dry dichloromethane (3.0 mL) over 10 min. After stirring at 0 °C for 15 min and then room temperature for 36 h the mixture was diluted with EtOAc (500 mL) and washed with 1 N $KHSO_4$ (3 × 350 mL), brine (350 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography (1/2 EtOAc/hexane) to give 4.36 g (52%) of **3**: TLC, R_f = 0.23 (1/2 EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 9H), 0.90−1.00 (m, 2H), 1.25−1.33 (m, 3H), 1.42 (s, 9H), 2.96−3.14 (m, 2H), 3.46 (s, 2H), 4.13−4.25 (m, 4H), 4.40−4.55 (m, 1H), 4.95−5.05 (m,-1H), 7.00−7.10 (m, 2H), 7.40−7.50 (m, 2H), 9.20 (s, 1H); ^{13}C NMR ($CDCl_3$) −1.0, 14.6, 17.9, 28.8, 38.2, 42.4, 55.1, 62.4, 64.3, 80.3, 120.6, 130.4, 132.8, 137.1, 155.7, 163.6, 170.2, 172.4. Anal. Calcd for $C_{24}H_{38}N_2O_7Si$: C, 58.28; H, 7.74; N, 5.66. Found: C, 58.05; H, 7.76; N, 5.57.

***N*-α-Boc-*β*-[*p*-[[Ethoxy-2-[(dimethylamino)methylene]-malonyl]amino]phenyl]-L-alanine 2-(Trimethylsilyl)ethyl Ester (4).** To a 250 mL reaction vessel was added 4.36 g (8.81 mmol) of **3**, 1.75 mL (13.2 mmol) of DMF dimethylacetal, and 20 mL of dry CH_2Cl_2. The reaction vessel was sealed with a rubber septum under argon and immersed in an oil bath heated to 55−60 °C for 40 h *(Caution: the boiling point of methylene chloride is 40 °C which will result in a pressure buildup inside the reaction vessel.* Shielding and a reaction vessel designed for running reactions under pressure should be used). The reaction was then concentrated followed by further drying under high vacuum to give 4.5 g (94%) of **4**: TLC, R_f = 0.05 (1/2 EtOAc/hexane), 0.21 (1/1 EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 0.04 (s, 9H), 0.94−1.00 (m, 2H), 1.32 (t, J = 6.9 Hz, 3H), 1.41 (s, 9H), 2.93−3.08 (m, 2H), 3.15 (br s, 6H), 4.15−4.22 (m, 4H), 4.48 (m, 1H), 4.94 (d, J = 8.1 Hz, 1H), 7.05 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 8.01 (br s, 1H), 10.13 (br s, 1H); FABMS (+) m/z = 550 (M + 1).

Attempted Oxidation of *N*-α-Boc-*β*-[*p*-[[Ethoxy-2-[(dimethylamino)methylene]malonyl]amino]phenyl]-L-alanine 2-(Trimethylsilyl)ethyl Ester (4) with Singlet Oxygen. A solution of 0.94 g (1.7 mmol) of enamine **4** and 5 mg (0.0038 mmol) of rose bengal bis(triethylammonium) salt (Aldrich) was prepared in anhydrous CH_2Cl_2 (10 mL). The solution was cooled to −78 °C and then a constant supply of oxygen was introduced by bubbling O_2 through the reaction via a needle. The reaction was then irradiated with a 500-W quartz halogen lamp. The reaction was irradiated for 45 min, but a TLC (1/1 EtOAc/hexane) analysis showed no reaction had occurred.

Low Yield Oxidation of *N*-α-Boc-*β*-[*p*-[[Ethoxy-2-[(dimethylamino)methylene]malonyl]amino]phenyl]-L-alanine 2-(Trimethylsilyl)ethyl Ester (4) with Ozone. A solution of 0.94 g (1.7 mmol) of enamine **4** in 20 mL of dry CH_2Cl_2 was prepared and cooled to −78 °C. Ozone was then continuously passed through the reaction by bubbling through a submerged needle for 45 min during which time the reaction became a light yellow-green. Dimethyl sulfide (3 mL) was then added to prevent further oxidation while the temperature was gradually increased to 25 °C. Stirring was continued for another 40 min. The solvent and dimethyl sulfide were removed under vacuum, and the residue was purified by flash chromatography (2/1 EtOAc/hexane) to give only 70 mg (8%) of the tricarbonyl compound **5** and 150 mg (16.7%) of its hydrated derivative **6**.

***N*-α-Boc-*β*-[*p*-[(Ethoxy-2-oxomalonyl)amino]phenyl]-L-alanine 2-(Trimethylsilyl)ethyl Ester (5) and Its Hydrate (6).** To a solution of enamine **4** (290 mg, 0.52 mmol) in 50% aqueous THF (10 mL) was added $NaIO_4$ (350 mg, 1.63 mmol). After 18 h at rt the starting material **4** was consumed as judged by TLC. The reaction was concentrated, the residue was diluted with EtOAc (5 mL), and the insoluble white precipitate was removed by filtration. The filtrate was concentrated and purified by flash chromatography (1/2 EtOAc/hexane containing 1% acetic acid) to give **5** (100 mg) and **6** (110 mg) in a 79% combined yield. Compound **5**: TLC, R_f = 0.65 (1/2 EtOAc/hexane containing 1% acetic acid); ^{1}H NMR($CDCl_3$) 0.02 (s, 9H), 0.90−1.00 (m, 2H), 1.30−1.42 (m, 12H), 2.90−3.20 (m, 2H), 4.10−4.22 (m, 2H), 4.23−4.30 (m, 2H), 4.40−4.60 (m, 1H), 4.90−5.00 (m, 1H), 7.05−7.15 (m, 2H), 7.45−7.50 (m, 2H), 8.50−8.70 (m, 1H). ^{13}C NMR ($CDCl_3$) −1.0, 14.8, 17.9, 28.9, 38.4, 55.1, 61.4, 64.3, 80.4, 121.49 & 121.62 (assigned as conformational isomers), 130.6, 133.5, 136.1, 158.9, 167.9, 169.5, 172.4, 178.2. Anal. Calcd for $C_{24}H_{36}N_2O_8Si$: C, 56.67; H, 7.13; N, 5.51. Found: C, 56.81; H, 7.14; N, 5.42. Compound **5** showed limited stability upon extended storage at rt. Compound **6**: TLC, R_f = 0.28 (1/2 EtOAc/hexane containing 1% acetic acid); ^{1}H NMR ($CDCl_3$) δ 0.02 (m, 9H), 0.95−1.00 (m, 2H), 1.30 (t, J = 6.8 Hz, 3H), 1.42 (s, 9H), 2.90−3.16 (m, 2H), 4.15−4.44 (m, 4H), 4.45−4.53 (m, 3H), 5.01 (d, J = 6.5 Hz, 1H), 7.11−7.18 (m, 2H), 7.47−7.50 & 7.56−7.61 (two m, 2H, assigned as conformational isomers), 8.30 & 8.55 (two s,

1H, assigned as conformational isomers). ^{13}C NMR ($CDCl_3$) −1.0, 14.49 & 14.56 (assigned as conformational isomers), 18.0, 28.9, 38.3, 55.1, 63.8, 64.24 & 64.45 (assigned as conformational isomers), 80.5, 91.7, 120.47 & 120.54 (assigned as conformational isomers), 130.66 & 130.80 (assigned as conformational isomers), 133.8, 134.70 & 136.05 (assigned as conformational isomers), 155.6, 166.2, 170.1, 172.35 & 172.41 (assigned as conformational isomers). Anal. Calcd for $C_{24}H_{38}N_2O_9Si$: C, 54.73; H, 7.27; N, 5.32. Found: C, 54.79; H, 7.24; N, 5.22.

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