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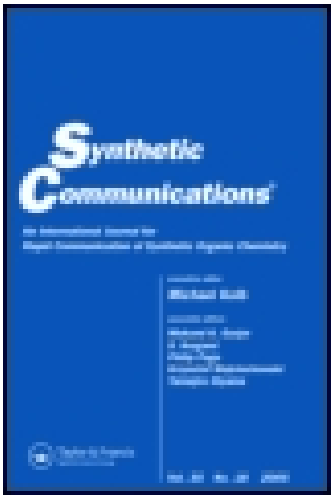

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A Convenient, Large Scale Synthesis of N-CBZ-(S-Phenyl)-L-Cysteine

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A CONVENIENT, LARGE SCALE SYNTHESIS OF N-CBZ-(S-PHENYL)-L-CYSTEINE

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Abstract: N-Cbz-(S-phenyl)-L-cysteine (**3**) has been prepared on a multi-kilogram scale in high yield and optical purity from the β-lactone of N-Cbz-L-serine.

(S-Phenyl)-L-cysteine (**1**) is a non-proteinogenic amino acid that has been synthesized at various times to investigate its utility as a potential anti-cancer agent[1], as a building block for β-lactam formation[2,3] and more recently as a

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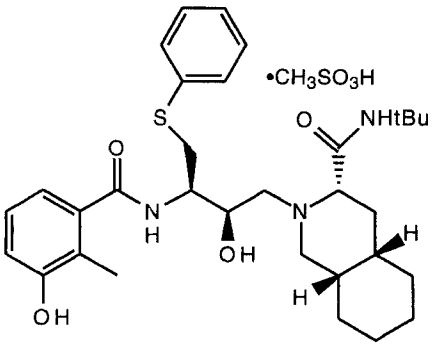
starting material for inhibitors of Kynureninase.[4] Our interest in **1** resulted from the discovery of AG-1343 (**2**), a novel HIV protease inhibitor currently undergoing clinical evaluation.[5]



2

Successful manufacture of **2** depends on a reliable and scalable synthesis of N-Cbz-(S-phenyl)-L-cysteine (**3**). A variety of methods to make **1** and its protected derivatives, such as **3**, have been reported previously in the literature. These include preparation from L-cysteine by copper catalyzed reaction with phenyl diazonium salts[2,6] or iodobenzene[7], from L-serine by enzyme catalysis[4] or by displacement[8], and by addition to a chiral Ni(II) complex of dehydroalanine.[9] Enantiomerically enriched (ee=98%) (S-phenyl)-L-cysteine has also been prepared by kinetic resolution[10] of racemic (S-phenyl)cysteine, available by several routes starting with achiral materials.[1,11] While successful to some degree, none of these methods are amenable to development at pilot or commercial scale. Either the starting materials are not available beyond research amounts, the yields are too low to be economically viable, or processing would present formidable challenges. Since our development needs required multi-kilogram quantities of **3**, a novel synthetic route was sought.

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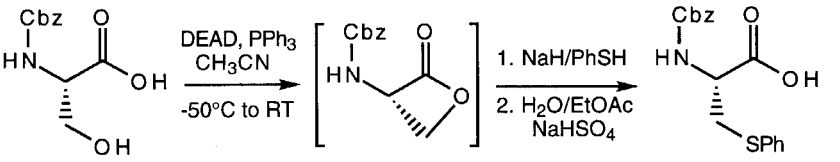
Vederas, et. al.[12] have reported numerous nucleophilic displacements on N-Cbz-L-serine β-lactone to yield a variety of N-protected α-amino acids in high optical purity. Initial application of this approach using thiophenol as nucleophile gave the desired **3**, but the reaction proceeded in limited yield, used dimethyl azodicarboxylate as a reagent, and required a chromatographic work-up. The fact, however, that the reagents required (or their equivalents) were available on a commercial scale invited further development efforts.



3

Elimination of the unscalable chromatographic workup used to purify the intermediate N-Cbz-L-serine β-lactone and replacement of dimethyl azodicarboxylate with the more readily available diethyl azodicarboxylate were our first goals. Small scale experiments in THF at -70°C demonstrated that the β-lactone formed from diethyl azodicarboxylate (DEAD), triphenylphosphine, and N-Cbz-L-serine could be converted to **3** without isolation. Separation of the desired product from diethyl hydrazinodicarboxylate (DEAD-H_2), triphenylphosphine oxide, unreacted N-Cbz-L-serine, and other side products remained the only barrier to scale-up. Using the cumbersome sequence of evaporation of the reaction mixture, recrystallization of the residue from absolute ethanol, followed by dissolution of the recovered solid in water, filtration of the insolubles, and extraction with methylene chloride, yielded a fairly pure aqueous solution of N-Cbz-(S-phenyl)-L-cysteine, sodium salt. Acidification of the water layer, followed by extraction with ethyl acetate and evaporation of the subsequent

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organic layer yielded a crude oil that could be triturated with cyclohexane to yield **3** in 30-35% yield. The product was generally contaminated with 5-15 mol percent of DEAD-H_2. Conversion of numerous 22L batches eventually yielded 3.5 kg of **3**.

On completion of the synthesis, reevaluation of the process suggested several avenues for improvement. Analysis of mother liquors showed that, while significant amounts of product were lost during ethanol recrystallization (which also removed triphenylphosphine oxide and DEAD-H_2), a substantial yield loss also occurred due to side products formed during the β-lactone ring closure. Vederas had reported[13] that switching from THF to CH_3CN as the reaction solvent significantly reduced side reactions during β-lactone formation. On implementation of this change, it was found that not only could the reaction be run at a warmer temperature (-40 to -30°C), but the product precipitated in very high yield as its sodium salt from the reaction mixture. Filtration and washing of the cake with fresh CH_3CN yielded a product essentially free of side products and triphenylphosphine oxide. The level of DEAD-H_2 was also greatly reduced. Dissolution of the wet filter cake in water yielded a solution free of undissolved solids. Extraction with methylene chloride removed the remaining DEAD-H_2. Acidification of the aqueous layer and extraction with ethyl acetate, followed by drying of the organic layer over magnesium sulfate and evaporation of the solvent yielded an oil that crystallized on standing to give an average 79.4% yield of **3**. The product typically assayed 94-96% pure by HPLC, contained 1-3 mol percent DEAD-H_2, and had an enantiomeric excess of >99%. Using this procedure we have been able to manufacture more than 20 kg of **3** in 22L flasks. The process is currently being scaled to larger equipment.

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EXPERIMENTAL

All chemicals were used as purchased. Drying of N-Cbz-L-serine, triphenylphosphine, and solvents, recommended by Vederas[12], was found not to improve the yield. Reactions were run under a nitrogen atmosphere. HPLC analysis of the product was performed on a Gilson model 306 gradient HPLC using a 250 mm X 4.6 mm Capcell C-18 silica column (flow rate=1.5 mL/min, mobile phase=0.01M H_3PO_4:CH_3CN, gradient 50>>55% CH_3CN over eight minutes, UV detector set at 210 nm). Optical rotations were measured on a Jasco DIP370 Polarimeter using a sodium lamp at λ=589. ^{1}H-NMR spectra were collected using a GE QE300 300MHz spectrometer in $CDCl_3$/TMS. All analyses were performed by Agouron Pharmaceuticals' QC department, except for elemental analysis which was conducted by Atlantic Microlab.

N-Cbz-(S-phenyl)-L-cysteine (**3**)

To a flame dried 22L three neck roundbottom flask, equipped with mechanical stirrer, addition funnel, thermometer, and nitrogen head was added 1097 g triphenylphosphine (4.18 mol, 1.00 eq) and 12L CH_3CN. This mixture was heated to 30°C to dissolve all solids, then cooled to -40°C in an acetone/dry ice bath. The triphenylphosphine partially precipitated during cooling. To this mixture was added 658 mL diethyl azodicarboxylate (4.18 mol, 1.00 eq) at such a rate as to maintain the reaction temperature below -35°C. This mixture was stirred for 10 min.

A solution of 1000 g N-Cbz-L-serine (4.18 mol, 1.00 eq) was prepared in 1500 mL CH_3CN to which 400 mL warm ($\approx$ 45°C) THF had been added. This solution

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was added to the cooled 22L flask at such a rate as to maintain the reaction temperature below -35°C. During this addition, the reaction mixture became very thick and taffy-like. After complete addition, cooling was removed and the mix allowed to warm towards ambient temperature. On continued stirring, a pale yellow solution of N-Cbz-L-serine β-lactone formed.

To a second 22L three neck roundbottom flask, equipped as above, was added 168 g sodium hydride, 60% dispersion in mineral oil (4.2 mol, 1.0 eq) and 2L THF. This suspension was cooled to 0°C in an ice bath and 430 mL thiophenol (4.19 mol, 1.0 eq) was added as a steady stream while maintaining reaction temperature below 10°C. The mixture was removed from the ice bath and stirred for several minutes until gas evolution ceased. To this mixture was added in one portion the β-lactone solution from above. The resultant gray solution was stirred overnight. By the next morning, a dense white precipitate had formed in the reaction vessel. The precipitate was collected by filtration and the filter cake rinsed with a minimum amount of fresh CH_3CN. After pulling as dry as possible, the wet filter cake was transferred to a 22L extraction flask and dissolved in 6L deionized water. The aqueous solution was extracted three times with 2L CH_2Cl_2, acidified with 4L of 1M $NaHSO_4$, and extracted again with three times with 3L EtOAc. The combined EtOAc layers were washed with 2L sat. NaCl solution and then filtered through a $MgSO_4$ plug. The EtOAc solution was evaporated and the resulting oil allowed to crystallize over night. The solid product was transferred to drying pans and the residual solvent removed by drying *in vacuo* at 40°C. After drying, 1123 g were obtained in 81% theory yield. The product assayed by HPLC to be 94.7% pure, $[\alpha]$=-51.7° (c=1.01% wt/vol in MeOH), m.p. 88.5°-91.5°C, ^1H NMR ($CDCl_3$/TMS): δ3.40 (2H, m), 4.61(1H, dq, J=3Hz, 7Hz), 5.56

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(1H, d, J=7 Hz), 5.07 (2H,s), 7.3 (10H, m), Anal. Calcd for $C_{17}H_{17}NO_4S$: C, 61.61; H, 5.17; N, 4.23; S, 9.68. Found C, 61.53; H, 5.14; N, 4.27; S, 9.62

Determination of Optical Purity

N-Cbz-(S-phenyl)-L-cysteine N-methyl-O-methylcarboxamide was made from **3** in quantitative yield by the method of Goel[14]. HPLC analysis of the amide made from racemic N-Cbz-(S-phenyl)cysteine using a 4 mm X 150 mm 5μ Chiral-AGP analytical column (manufactured by ChromTech, available from Regis Technologies, Morton Grove, Ill) gave baseline resolution of the two enantiomers (flow rate=1.0 mL/min, mobile phase=20% CH_3CN/80% 0.015M Na_2HPO_4 and 0.015M KH_2PO_4, pH adjusted to 4 with H_3PO_4, UV detector at 210 nm). Analysis of the N-methyl-O-methylcarboxamide of **3** showed relative peak areas of 99.6% and 0.2% for the two enantiomers for an ee=99.4%.

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