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Regioselective nitration of N^{α},N^{1}-bis(trifluoroacetyl)-L-tryptophan methyl ester: Efficient synthesis of 2-nitro and 6-nitro-*N*-trifluoroacetyl-L-tryptophan methyl ester

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ABSTRACT

Nitration of N^{α},N^{1}-bis(trifluoroacetyl)-L-tryptophan methyl ester with HNO_3 in acetic anhydride at 0 °C provides N^{α}-trifluoroacetyl-2-nitro-L-tryptophan methyl ester in 67% yield, whereas nitration in trifluoroacetic acid at 0 °C gives N^{α}-trifluoroacetyl-6-nitro-L-tryptophan methyl ester in 69% yield.

Tryptophan is the largest and rarest of the genetically encoded amino acids. In addition to its role in proteins, L-tryptophan is also the precursor to a wide range of biologically active compounds, including serotonin, melatonin, kynurenine, NAD^+ (via quinolinate), and a host of indole alkaloids. Bromotryptophans are common components in toxic peptides or peptide-derived natural products from marine organisms.[1] Furthermore, metabolism of L-tryptophan by indoleamine-2,3-dioxygenase has been found recently to protect the fetus from rejection by the mother and regulate the human immune response.[2] For these reasons, substituted analogs of L-tryptophan have been the subject of considerable chemical and biological interest. A wide range of L-tryptophan analogs have been prepared by either chemical[3] or enzymatic synthesis.[4] However, it is attractive to be able to derivatize the indole ring of L-tryptophan directly, thus avoiding a resolution step and increasing efficiency by reducing the number of reactions. There are relatively few practical reactions for performing substitution reactions on L-tryptophan or a suitably protected derivative. Nitration is an attractive reaction, since nitro products are easily converted to a wide range of derivatives via reduction followed by Sandmeyer and related reactions. For example, N^{α}-trifluoroacetyl-6-nitro-L-tryptophan methyl ester was used to prepare 6-azido-L-tryptophan for photoaffinity labeling of tryptophan synthase from *Salmonella typhimurium*.[5] Direct nitration of L-tryptophan as the nitrate salt affords 6-nitro-L-tryptophan in modest yield.[6] In our hands, direct nitration of L-tryptophan under the reported conditions gives lower yield, involves large volumes of glacial acetic acid, and requires a messy workup. An improved nitration procedure of D-tryptophan using red fuming nitric acid in glacial acetic acid was reported,[7] but the higher yields were also not reproducible with L-tryptophan in our hands. We have reported previously that nitration of the protected L-tryptophan derivative, N^{α}-trifluoroacetyl-L-tryptophan methyl ester, in a mixture of nitric and acetic acid affords the 2-nitro product (**2**) in 6.8% yield and the 6-nitro product (**3**) in 40% isolated yield.[8] However, a complex product mixture is obtained, requiring tedious column chromatography for purification. In contrast, reaction of N^{α}-trifluoroacetyl-L-tryptophan methyl ester with NBS in CCl_4 yields the protected 2-bromotryptophan derivative in 83% yield, and the corresponding protected 2-chlorotryptophan can be obtained in high yield under the same conditions with NCS.[9]

Thus, we were interested in another suitably protected derivative of L-tryptophan for potential use in nitration and other electrophilic aromatic substitution reactions. In a search of the literature, we found that N^{α},N^{1}-bis(trifluoroacetyl)-L-tryptophan methyl ester (**1**) was reported in 1965 as a tryptophan derivative for gas chromatographic analysis of amino acids.[10] However, while the synthesis was described, no spectroscopic characterization of **1** was reported, and we found no subsequent reports on the use of this compound as a substrate for nitration or other electrophilic aromatic substitutions. The addition of a trifluoroacetyl group to the indole nitrogen affects the electronic environment of the aromatic system, deactivating the pyrrole ring and potentially allowing for selective substitution on either the benzene or pyrrole rings.

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Synthesis of **1** was performed in a two step procedure similar to that reported by Makisumi and Saroff.[10] First, L-tryptophan was converted to the methyl ester hydrochloride by treatment with $SOCl_2$ and methanol at −42 °C. L-Tryptophan methyl ester hydrochloride was then reacted with excess trifluoroacetic anhydride at room temperature for 3 h, followed by precipitation with petroleum ether to afford the N^{α},N^{1}-bis(trifluoroacetyl)tryptophan product (**1**) in 44% overall yield as sparkling white needles. The NMR data confirmed the structure of **1**.

We were pleased to find that nitration of **1** occurred under a variety of conditions, and regioselectivity for either the 2-nitro (**2**) or 6-nitro product (**3**) was achieved by appropriate choice of reaction solvent (Scheme 1). Acetic acid and acetic anhydride are common solvents for nitration of electron-rich heterocyclic substrates such as indoles, pyrroles, and thiophenes. Acetic acid, acetic anhydride, trifluoroacetic acid, and trifluoroacetic anhydride were compared in reactions of **1** with nitric acid at 0 °C, 25 °C, and 60 °C (Table 1). The reactions were worked up with water, extracted with EtOAc, and analyzed by GC–MS. In contrast to our earlier work with $N^{\alpha}N^{\alpha}$-trifluoracetyl-L-tryptophan methyl ester,[8] nitration of **1** in acetic acid, even for 24 h at 25 °C, did not produce significant amounts of **2** or **3** (Experiment 1, Table 1). However, nitration in trifluoroacetic acid produced the 6-nitro product (**3**) in 69% yield at both 0 °C for 1 h and 25 °C for 15 min (Experiments 3 and 7, Table 1). Incubation at 60 °C resulted in extensive degradation (Experiments 9 and 10, Table 1). Despite similar yields by GC, the product afforded by the reaction at 0 °C proved much easier to purify. In contrast, nitration of **1** in acetic anhydride at 0 °C for 1 h afforded predominantly the 2-nitro derivative (**2**) in 67% yield (Experiment 6, Table 1). The analytical scale reactions were worked up simply by diluting the product into distilled water and extracting the organic products into ethyl acetate, which kept all protecting groups intact, as determined by GC–MS.

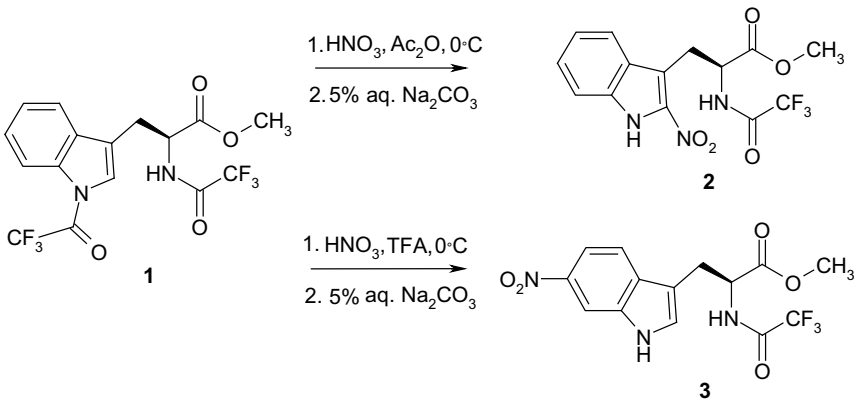
Hydrolysis of the reaction products, **2** and **3**, to the free 2- and 6-nitrotryptophans, respectively, was performed as described previously.[8] The 2- and 6-nitrotryptophans were then analyzed for optical purity by HPLC on a 4 × 250 mm Chiral ProCu = Si100Polyol 3-hydroxy-D-proline column (Serva) with 5 mM $CuSO_4$, pH 3.15, at 1 ml/min. HPLC analysis of the corresponding DL-amino acids on the chiral column showed two well separated peaks. No measurable amount of the D-isomer was detected in the 2- and 6-nitro-L-tryptophans, indicating that the nitrotryptophan products are greater than 98% homochiral. This demonstrates that no significant tryptophan racemization has taken place during the trifluoroacetylation or nitration reactions.

Selective nitration of the 2-position in the neutral solvent, acetic anhydride, is a reflection of the inherently higher reactivity of the pyrrole ring of indole with electrophiles, possibly acetyl nitrate in this case. In support of this interpretation, the nitration of *N*-phenylsulfonylindole with acetyl nitrate was reported to exhibit temperature dependent regioselectivity, with the 3-nitroindole product favored at low temperatures, but the 6-nitroindole product increasing at temperatures above −10 °C.[11] The regioselectivity for 6-nitration that we observe in trifluoroacetic acid is due to the higher reactivity of the electrophile, nitronium ion, derived from either nitric acid itself, or perhaps trifluoroacetyl nitrate, in the strongly acidic medium, directing the site of nitration to the less reactive but less hindered 6-position in the benzene ring. We note that 2-nitroindoles are generally difficult to prepare, especially in high yields, and the best previously reported procedure to prepare 2-nitroindoles by nitration is via lithiation of *N*-phenylsulfonyl or *N*-Boc-indoles with *tert*-butyl lithium, followed by reaction with N_2O_4 in frozen THF at −120 °C to give 63–78% yields.[12]

Table 1
Nitration experiments with N^{α},N^{1}-bis(trifluoroacetyl)-L-tryptophan methyl ester

Experiment	Temp. (°C)	Solvent	Time	**1**, %	**2**, %	**3**, %	other, %
1	25	CH_3COOH	24 h	58	0	0	42
2	25	$(CH_3CO)_2O$	15 min	40	33	15	12
3	25	TFA	15 min	0	12	67	21
4	25	$(TFA)_2O$	15 min	0	4	8	88
5	0	CH_3COOH	24 h	45	2	3	50
6	0	$(CH_3CO)_2O$	1 h	0	67	14	19
7	0	TFA	1 h	0	4	69	27
8	0	$(TFA)_2O$	4 h	9	7	10	74
9	60	$(CH_3CO)_2O$	4 h	0	6	12	82
10	60	TFA	4 h	0	0	10	90

Gram scale syntheses of **2** and **3** were achieved by quenching the reaction mixtures in 5% aqueous sodium carbonate, filtering the precipitated product, and purification by recrystallization.[13] No chromatography was required. Workup in 5% aqueous sodium carbonate allowed for a higher isolated yield, but with the concomitant cleavage of the N^{1}-trifluoroacetyl group from the indole. The NMR spectra of **2** and **3** thus obtained were identical to those reported previously.[8] For some purposes it may be desirable to isolate the bis(trifluoroacetyl) product. The N^{1}-trifluoroacetyl group can be retained by diluting the reaction mixture with water, followed by extraction into ethyl acetate; however, the bis(trifluoroacetyl) products do not recrystallize as efficiently, and the labile N^{1}-trifluoroacetyl group cleaves from the nitro product during flash chromatography on silica gel, resulting in mixtures of the mono and bis(trifluoroacetyl) nitro-products. In contrast to the nitration reactions, bromination reactions of **1** with a variety of reagents (Br_2, NBS), solvents (acetic acid, CCl_4) and temperatures were attempted, but all reactions were unsuccessful and gave only recovered starting material.



Scheme 1. Nitration reactions of N^{α},N^{1}-bis(trifluoroacetyl)-L-tryptophan methyl ester.

In conclusion, nitration of N^{α},N^{1}-bis(trifluoroacetyl)-L-tryptophan methyl ester occurs efficiently at 0 °C to provide high yields of either the 2-nitro or 6-nitro product, depending on the choice of reaction solvent as acetic anhydride or trifluoroacetic acid, respectively. The workup conditions can result in either the mono or bis(trifluoroacetyl)-protected nitrotryptophan product. This reaction gives a much higher yield of protected 2-nitrotryptophan than our previous procedure, and yields comparable or better than the other published procedures for 6-nitrotryptophan.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.09.086.

References and notes

1. Bittner, S.; Scherzer, R.; Harlev, E. *Amino Acids* **2006**, *33*, 19.
2. (a) Munn, D. H.; Zhou, M.; Attwood, J. T.; Bondarev, I.; Conway, S. J.; Marshall, B.; Brown, C.; Mellor, A. L. *Science* **1998**, *281*, 1191; (b) Mellor, A. L.; Munn, D. H. *Immunol.Today* **1999**, *20*, 469.
3. (a) Lee, M. S.; Phillips, R. S. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 477; (b) Dua, R. K.; Phillips, R. S. *Tetrahedron Lett.* **1992**, *33*, 29; (c) Ma, C.; Liu, X.; Li, X.; Flippen-Anderson, J.; Yu, S.; Cook, J. M. *J. Org. Chem.* **2001**, *66*, 4525.
4. (a) Sloan, M. J.; Phillips, R. S. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1053; (b) Lee, M.; Phillips, R. S. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1563; (c) Welch, M.; Phillips, R. S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 637; (d) Phillips, R. S. *Tetrahedron: Asymmetry* **2004**, *15*, 2787.
5. Miles, E. W.; Phillips, R. S. *Biochemistry* **1985**, *24*, 4694.
6. De Fazi, R.; Berti, G.; Da Settimo, A. *Gazz. Chim. Ital.* **1959**, *89*, 2238.
7. Moriya, T.; Hagio, K.; Yoneda, N. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2217.
8. Phillips, R. S.; Cohen, L. A. *J. Heterocycl. Chem.* **1988**, *25*, 191.
9. (a) Phillips, R. S.; Cohen, L. A. *Tetrahedron Lett.* **1983**, *24*, 5555; (b) Phillips, R. S.; Cohen, L. A. *J. Am. Chem. Soc.* **1986**, *108*, 2023.
10. Makisumi, S.; Saroff, H. A. *J. Gas Chromatogr.* **1965**, *1*, 23.
11. Pelkey, E. T.; Gribble, G. W. *Synthesis* **1999**, 1117.
12. Jiang, J.; Gribble, G. W. *Tetrahedron Lett.* **2002**, *43*, 4115.
13. In a typical reaction, N^{α},N^{1}-bis(trifluoroacetyl)-L-tryptophan methyl ester (2.0 g, 4.84 mmol) was placed in a 50 ml round bottomed flask with 10 ml of solvent, either acetic anhydride or trifluoroacetic acid. The reaction mixture was cooled to −2 °C in an ice-salt bath. A solution of 70% nitric acid (3 ml) in the same solvent (10 ml) was added drop-wise and the reaction was stirred. After 45 min, the reaction was quenched by pouring into ice-cold 5% aqueous sodium carbonate solution. Additional sodium bicarbonate was added until the pH of the mixture was 7.5. The resulting yellow precipitate was collected by filtration, and the cake was washed with cold water. The dried product was dissolved in a minimal volume of ethyl acetate and crystallized by addition of hexane. After standing at 4 °C overnight, the product was collected by filtration, washed with a small volume of hexanes and air-dried.