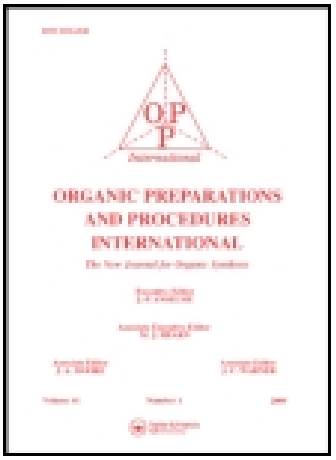

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An Improved Protocol for the Synthesis of 1-(Mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT)

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OPPI BRIEF

An Improved Protocol for the Synthesis of 1-(Mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT)

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Since the pioneering work of Merrifield,[1–5] solid-phase synthesis has become a key technology for the rapid parallel synthesis of polypeptides and other oligomeric compounds for drug discovery and chemical biology research. With an increasing number of peptide and peptide mimetic drug candidates in the pipelines of large pharmaceutical companies, solid-phase synthesis will likely continue to have a major impact on pharmaceutical chemistry.

For solid-phase synthesis, it is essential that all transformations on the solid support proceed in a quantitative fashion. Until the final step of a synthetic sequence, reaction products remain covalently bound to the support and can only be purified by simple washings and filtrations, rendering any incomplete reaction step along the way a source of decreased yields of products.

Solid-phase synthesis typically involves the creation of amide- or ester bonds, either as part of the linkage strategy, or to build the final product. In this context, a multitude of coupling reagents has been developed. For example, reagents for esterification on solid support include *N,N′*-dicyclohexylcarbodiimide (DCC) in conjunction with 4-(*N*,*N*-dimethylamino)pyridine (DMAP)[6] or 1-hydroxybenzotriazole (HOBt),[7] 2,6-dichlorobenzoyl chloride,[8] and diethyl azodicarboxylate–triphenylphosphine.[9] However, these activators generally result in inferior conversions and product yields. 1-(Mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) has most often been used for the formation of phosphate- and phosphorothiolate esters in oligonucleotide synthesis,[10–18] and, to a limited extent for amide-bond formation in peptide synthesis.[19,20] Another important application of MSNT is its usefulness to anchor the C-terminal of amino acids to hydroxyl-functionalized supports.[21–23] MSNT-based esterification protocols generally proceed with minimal racemization in high yields, and have therefore frequently been

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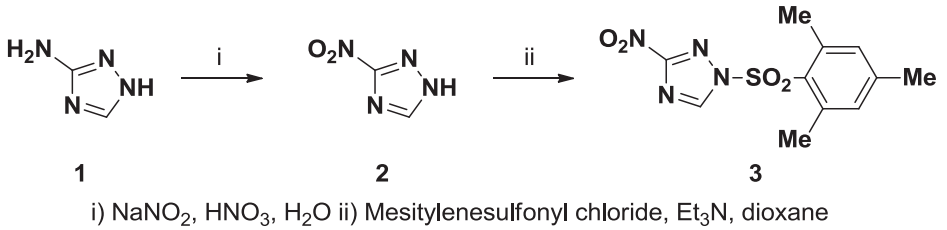
employed for the solid-phase synthesis of peptides, glycopeptides, and peptidomimetics.[24–36] It is therefore desirable that the reagent be obtainable in a scalable, efficient, and reliable manner. The only method known[37] describes the preparation of MSNT in 63% yield in two steps from 3-amino-1,2,4-triazole, through the intermediacy of 3-nitro-1,2,4-triazole (**2**). This intermediate can be prepared from 3-amino-1,2,4-triazole (**1**) using sodium nitrite/acetic acid,[38] sodium nitrite/acetic acid/sulfuric acid,[39] sodium nitrite/sulfuric acid[40] or hydrogen peroxide/trifluoroacetic acid,[41] sodium tungstate/hydrogen peroxide,[42] sodium perborate/acetic acid.[43] However, the yields of these procedures (23%–77%) are either lower than or similar to that reported by Jones *et al*.[37] The only other method using sodium nitrite/hydrochloric acid[44] reported to give a higher yield (87%) was not reproducible in our hands. Starting with commercial 3-amino-1,2,4-triazole (Sigma-Aldrich), we now describe a reproducible, scalable, and highly practical protocol for the synthesis of MSNT.

In the first step, 3-amino-1,2,4-triazole (~25 g) is diazotized by slow addition of reagent in a 2 l round-bottomed flask under mechanical stirring. The slow rate of addition, the use of a large volume flask and mechanical stirring are experimental parameters that were carefully chosen to overcome the occurrence of extensive foaming and precipitation. The product (3-nitro-1,2,4-triazole) was subsequently collected and purified by recrystallization in 62%–68% yields. The generation of highly toxic NO_x gases requires the use of a well-ventilated fume hood. In the second step, 3-nitro-1,2,4-triazole (~20 g) is sulfonylated using mesitylenesulfonyl chloride to give MSNT (*Scheme 1*), which is isolated in 46%–48% yield after extraction and purification by recrystallization. The purity of both the intermediate and the product can be determined by routine NMR and RP-HPLC techniques. In summary we have developed a practical synthesis of MSNT in 29%–33% overall yield, which is safer and more reproducible than previously reported methods.



Scheme 1

Experimental Section

All reagents and materials used were purchased commercially and were used without purification. The solvents used were of standard HPLC grade. Thin-layer chromatography was performed on aluminum plates, pre-coated with silica gel (Merck 25, 20 × 20 cm, 60 F_{254}) using the indicated eluent systems. Visualization was carried out by illumination with a UV-lamp (254 nm). Flash column chromatography was performed using silica gel (FLUKA 60758 silica gel 60, particle size 0.035–0.070 mm, 220–440 mesh ASTM) in

varying sizes of glass columns equipped with a sintered glass filter, a joint pressure inlet, and a stopcock.

Melting points were measured using a Buch & Holm A/S melting point apparatus and are uncorrected. 1D NMR spectra were recorded using a Varian Mercury-300 MHz instrument in DMSO-d_6 or $CDCl_3$ using the residual $CHCl_3$ or DMSO solvent peaks, respectively, as the internal standards. All ^{13}C NMR spectra were proton decoupled. IR analyses were carried out on a Bruker Alpha FT-IR spectrometer. Products were analyzed on a Waters Alliance reverse-phase HPLC system consisting of a Waters 2695 Separations Module equipped with a Symmetry C_{18} column (3.5 μm, 4.6 $\times$ 75 mm, column temp 25°C, flow rate 1 ml/min) and a Waters Photodiode Array Detector (detecting at 215 nm). Elution was carried out in a linear reversed phase gradient fashion combining water and acetonitrile (buffered with 0.1% trifluoroacetic acid).

3-Nitro-1,2,4-triazole (2). A 2.0 l, three-necked, round-bottomed flask equipped with an overhead mechanical stirrer, a 100 ml pressure-equalizing addition funnel, and a glass-stopper was charged with 3-amino-1,2,4-triazole (26.3 g, 0.297 mol) and an aqueous sodium nitrite solution (100.0 g, 1.45 mol in 150 ml water) in an efficient fumehood. The suspension was cooled in an ice-water bath and the mechanical stirrer was started. After 10 min of stirring, the light suspension was treated dropwise with conc. nitric acid (85 ml) via the addition funnel over a period of 2.5–3.0 h (*foaming*).[45] After complete addition of nitric acid, the ice-water bath was removed and the yellow suspension was stirred for additional 1.0 h at room temperature until foaming stopped.[46] The crude product was collected and the yellow solid filter cake was dried overnight under oil pump vacuum, to give a yellow solid (47 g). The crude product was dissolved in boiling methanol (300 ml) for 30 min, and then filtered hot using house vacuum. The methanol filtrate was allowed to cool to rt. and then placed overnight in a freezer at −15°C whereupon 3-nitro-1,2,4-triazole crystallized as a light yellow solid, which was collected, washed with ice-cold methanol, and dried overnight at 1.9 mmHg to afford 21–23 g (62%–68%) of the title compound, mp. 208°C–211°C (*lit.*[37] 208–210).^1H NMR (300 MHz, DMSO-d_6): δ 8.86 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.1, 146.3; IR (solid): 3162, 2861, 2776, 2730. The purity of the product (97%) established by RP-HPLC, t_R = 2.01 min (254 nm).

1-(Mesitylenesulfonyl)-3-nitro-1,2,4-triazole (3). An oven dried 1.0 l round-bottomed flask containing a magnetic stir bar was equipped with a Claisen adapter. 3-Nitro-1,2,4-triazole (19.5 g, 0.171 mol), dry dioxane (200 ml) and triethylamine (1.0 equiv., 17.3 g, 0.171 mol) was transferred to the reaction flask and the solution was cooled in an ice-bath with magnetic stirring.[47] The Claisen adapter was fitted with a 200 ml pressure-equalizing addition funnel, containing a dioxane solution (150 ml) of mesitylenesulfonyl chloride (37.4 g, 0.171 mol). The solution of mesitylenesulfonyl chloride was added dropwise over a period of approx. 0.5 h and the final suspension was stirred for an additional 1 h and then warmed to rt. After removal of the precipitated $Et_3N{\cdot}HCl$ by filtration, the filtrate was concentrated *in vacuo* to give a yellow solid, which was dissolved in dichloromethane (150 ml) and the solution was washed with water (150 ml).[48] The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to give a yellow solid. Recrystallization from boiling toluene (20–30 ml) followed by washing with ice-cold toluene and drying overnight at 1.9 mmHg, provided 24–25 g (46%–48%) of the title compound as light yellow

solid,[49] mp. 131°C–133°C (*lit.*[37] 130°C–132°C). $R_f = 0.23$ (20% hexane in CH_2Cl_2, UV); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.84 (s, 1H), 7.07 (s, 2H), 2.69 (s, 6H), 2.34 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.9, 147.5, 145.2, 142.5, 133.0, 127.9, 23.2, 21.3; IR (solid): 3126, 2983, 2947, 1597.

Anal. Calcd. for $C_{11}H_{12}N_4O_4S$: C, 44.59; H, 4.08; N, 18.91. Found: C, 44.69; H, 3.88; N, 18.72. The purity of the product (96%) established by RP-HPLC, $t_R = 8.41$ min (254 nm).

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45. During the addition, extensive foaming is produced and the time of the addition should therefore be controlled to a minimum of 2.5 h.

46. The reaction mixture can be stirred overnight without affecting the yield.

47. When the reaction is performed on 10 g or less, the solvent may crystallize and this may lead to the complete shutdown of the reaction. The result of the use of THF as solvent was inferior compared to dioxane; the use of THF as co-solvent to alleviate this problem was not investigated.

48. During the wash, minor amounts of a solid is formed between the two phases. It is extremely important that the volume of water be carefully controlled and that only one wash be performed. If too much water is used or a second wash is carried out, the yield drops dramatically (to about 20%), possibly due to hydrolysis of the product.

49. Additional product ($\geq$10%) may be obtained by silica gel flash column chromatography (20% hexane in CH_2Cl_2) of the concentrated mother liquor from the crystallization.