Heteroarene-2-sulfonyl Chlorides (BtsCl; ThsCl): Reagents for Nitrogen Protection and >99% Racemization-Free Phenylglycine Activation with $SOCl_2$

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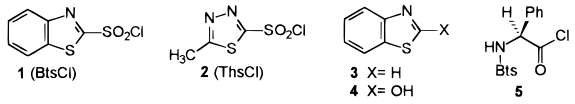
Acid chlorides derived from N-acylamino acids undergo facile cyclization to the easily racemized oxazolinones.[1] Nitrogen protection using alkoxycarbonyl groups improves amino acid chloride stability and the *N*-(fluorenylmethoxy)carbonyl (FMOC) derivatives can be stored in crystalline form.[1b] However, they can still cyclize to oxazolinones, especially if tertiary amines are present.[2] The corresponding acid fluorides may offer a better combination of reactivity and stability for use in peptide synthesis.[3,4] For applications that demand the reactivity of an acid chloride, the risk of oxazolinone formation can be avoided by using an arenesulfonyl group to protect nitrogen,[5,6] but arenesulfonamide cleavage in the amino acid series has been difficult. We report a solution to this problem using the heteroarenesulfonyl chlorides benzothiazole-2-sulfonylchloride (**1**, BtsCl, "betsyl" chloride) or 5-methyl-1,3,4-thiadiazole-2-sulfonyl chloride (**2**, ThsCl, "thisyl" chloride).



Commercially available 2-mercaptobenzothiazole or 2-mercapto-5-methyl-1,3,4-thiadiazole were treated with excess chlorine in HOAc−H_2O.[7] The resulting crystalline **1** or **2** were stable for months in the freezer, but evolution of SO_2 was apparent after several days or less at rt (room temperature), depending on crystal quality. Simple amines reacted rapidly with **1** and **2** under aprotic conditions. With zwitterionic amino acids, the best results were achieved by stirring a suspension of **1** or **2** in a solution of the amino acid in NaOH−H_2O while maintaining pH in the range of 10−10.5. This method afforded crystalline *N*-Bts and *N*-Ths derivatives in 92−97% yield with alanine, valine, phenylalanine, proline, and phenylglycine (Phg).[8] The *N*-Ths derivatives formed significantly faster (2 h at 0 °C for Ths-Phg vs 10 h at 10 °C for Bts-Phg), probably because ThsCl has greater water solubility compared to BtsCl. Under similar conditions, but using 3.1 equiv of ThsCl, both amino groups of lysine were protected (N_α,N_ϵ-bis-Ths-Lys, 89%). Serine reacted selectively using 1.5 equiv of ThsCl (74% *N*-Ths-Ser isolated),[9] but similar treatment with BtsCl afforded a mixture. The standard method produced the mono-Bts derivative of tryptophan (>95% yield) without affecting the indole nitrogen. Tyrosine gave the bis-sulfonylation product using 3.1 equiv of ThsCl at pH 10 (90%), but O-sulfonylation could be prevented by temporary O-silylation. Thus, heating with bis-(trimethylsilyl)acetamide (2.1 equiv, CH_3CN) followed by reaction with BtsCl in pyridine afforded *N*-Bts-Tyr in 72% yield. Modified nonaqueous conditions were also used to prepare Bts-Phg from Phg + Me_3SiCl/Et_3N (THF, reflux) followed by BtsCl/Et_3N to give Bts-Phg in 87% yield. Useful bis-N-sulfonylation of arginine was not achieved under a variety of conditions, however.

Removal of the *N*-Bts or *N*-Ths groups was accomplished at rt by treatment with Zn/HOAc−EtOH or Al−Hg/ether−H_2O. Slow addition of excess 50% H_3PO_2 to a ca. 1 M DMF solution of the substrate also worked, although removal of the DMF was tedious. No cleavage was observed in THF at rt, but good results were obtained by slow addition of 50% H_3PO_2 to the substrate in refluxing THF (for example, N_α-Bts-Trp to Trp; 90% isolated). Other reducing agents ($Na_2S_2O_4$ or $NaHSO_3$) removed the Bts group of *N*-Bts-Phg in refluxing EtOH−H_2O. The most convenient Zn, Al−Hg, or H_3PO_2 conditions did not cleave *p*-toluenesulfonamides in control experiments, indicating that the electron-withdrawing heterocycle activates the sulfone for reduction.[10] Deprotection of *N*-Bts-Phg with Zn/HOAc−EtOH at rt (14 h) produced Phg, in quantitative yield, 99.9% ee by chiral stationary phase (CSP) HPLC assay after conversion to the 3,5-dinitrobenzamide (DNB). Deprotection by the dropwise addition of excess 50% aqueous H_3PO_2 at 50 °C over 2 h gave Phg with 99.5% ee (93% yield). Benzothiazole (**3**) was formed as the byproduct of reductive cleavage and was easily separated by organic extraction. Deprotection of *N*-Ths-Phg also proceeded smoothly (>99.8% ee using Zn−HOAc/EtOH, Zn−HCl/THF, Al−Hg/THF−H_2O, >90% yield). The Bts group of Bts-Phg easily survived conditions that cleave BOC (CF_3CO_2H, 2 days at rt) or FMOC (Et_2NH, DMF, 21 h, rt). Slow cleavage did occur in CF_3CO_2H/C_6H_5SH (ca. 25%, 2 days rt), and Cbz hydrogenolysis conditions (H_2, Pd/C, EtOAc) resulted in partial Bts cleavage and catalyst poisoning. Deprotection with NaOH was effective for *N*-Bts-Pro (2.5 M NaOH: rt, 12 h, >99.8% ee by CSP HPLC after conversion to DNB-Pro-OMe; neutral byproduct 2-hydroxybenzothiazole, **4**), but harsher conditions were needed for *N*-Bts-Phg (1 M NaOH: 90−100 °C, 24 h; 14% ee). Finally, N_α-Bts-Trp-Met-Asp-Phe-NH_2 (protected cholecystokinin C-terminal tetrapeptide) was cleaved using slow addition of 50% H_3PO_2 in DMF at rt.[11]

(1) (a) For reviews, see: Kemp, D. S. In *The Peptides*; Gross, E., Meienhofer, J., Eds.; Academic Press, Inc.: New York, 1979; Vol. 1, p 315. Beyermann, M.; Granitza, D.; Bienert, M.; Carpino, L. A. In *Peptides: Chemistry and Biology*; Marshall, G. R., Ed.; ESCOM: Leiden, The Netherlands, 1988; p 101, 189. Bodanszky, M. *Principles of Peptide Synthesis*, 2nd ed.; Springer-Verlag: Berlin, 1993. (b) Carpino, L. A.; Cohen, B. J.; Stephens, K. E., Jr.; Sadat-Aalaee, S. Y.; Tien, J.-H.; Langridge, D. C. *J. Org. Chem.* **1986**, *51*, 3732. Frerot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259. Benoiton, N. L.; Lee, Y. C.; Steinaur, R.; Chen, F. M. F. *Int. J. Pept. Protein Res.* **1992**, *40*, 559. Spencer, J. R.; Antonenko, V. V.; DeLaet, N. G. J.; Goodman, M. *Int. J. Pept. Protein Res.* **1992**, *40*, 282. Akaji, K.; Kuriyama, N.; Kiso, Y. *Tetrahedron Lett.* **1994**, *35*, 3315.

(2) Carpino, L. A.; Chao, H. G.; Beyerman, M.; Bienert, M. *J. Org. Chem.* **1991**, *56*, 2635. Sivanandaiah, K. M.; Suresh Babu, V. V.; Shankaramma, S. C. *Int. J. Pept. Protein Res.* **1994**, *44*, 24.

(3) (a) Carpino, L. A.; Mansour, E.-S. M. E.; Sadat-Aalaee, D. *J. Org. Chem.* **1991**, *56*, 2611. (b) Carpino, L. A.; El-Faham, A. *J. Am. Chem. Soc.* **1995**, *117*, 5401. (c) Wenschuh, H.; Beyermann, M.; Krause, E.; Brudel, M.; Winter, R.; Schumann, M.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1994**, *59*, 3275. Carpino, L. A.; Sadat-Aalaee, D.; Chao, H. G.; DeSelms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651. (d) Carpino, L. A.; Mansour, E.-S. M. E.; El-Faham, A. *J. Org. Chem.* **1993**, *58*, 4162.

(4) (a) Kearns, J.; Kayser, M. M. *Tetrahedron Lett.* **1994**, *35*, 2845. (b) Xi, N.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36*, 6595. (c) Weisz, I.; Roboz, J.; Bekesi, J. G. *Tetrahedron Lett.* **1996**, *37*, 563.

(5) (a) Fischer, E. *Chem. Ber.* **1915**, *48*, 93. (b) Cleavage of *N*-(toluenesulfonyl)amino protecting groups: Maurer, P. J.; Takahata, H.; Rapoport, H. *J. Am. Chem. Soc.* **1984**, *106*, 1095. Hamada, T.; Nishida, A.; Yonemitsu, O. *J. Am. Chem. Soc.* **1986**, *108*, 140. Art, J. F.; Kestemont, J. P.; Soumillion, J. Ph. *Tetrahedron Lett.* **1991**, *32*, 1425. Roemmele, R.; Rapoport, H. *J. Org. Chem.* **1988**, *53*, 2367. (c) Vedejs, E.; Lin, S. *J. Org. Chem.* **1994**, *59*, 1602.

(6) (a) Carpino, L. A.; Shroff, H.; Triolo, S. A.; Mansour, E.-S. M. E.; Wenschuh, H.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 7829. Arzeno, H. B.; Kemp, D. S. *Synthesis* **1988**, 32. Hamada, T.; Nishida, A.; Yonemitsu, O. *Tetrahedron Lett.* **1989**, *30*, 4241. (b) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373. Coulaouic-Dubois, C.; Guggisberg, A.; Hesse, M. *J. Org. Chem.* **1995**, *60*, 5969.

(7) (a) Roblin, R. O., Jr.; Clapp, J. W. *J. Am. Chem. Soc.* **1950**, *72*, 4890. (b) Stanovnik, B.; Tišler, M. *Arch. Pharm.* **1965**, *298*, 357.

(8) (*R*)-Phenylglycine (0.483 g, 3.20 mmol) was dissolved in 0.25 M aqueous NaOH (11 mL, 2.8 mmol) at 10 °C. Solid **1** (1.10 g, 4.72 mmol) was added, and the suspension was stirred for 10 h at 10 °C while the pH was monitored and 1.3 M NaOH was added to maintain pH 10−10.5 (1.09 g, 97%), mp 162−165.5 °C (dec) after acidification and recrystallization.

(9) Product is water soluble; good yield requires evaporation of water.

(10) Kende, A. S.; Mendoza, J. S. *Tetrahedron Lett.* **1990**, *31*, 7105.

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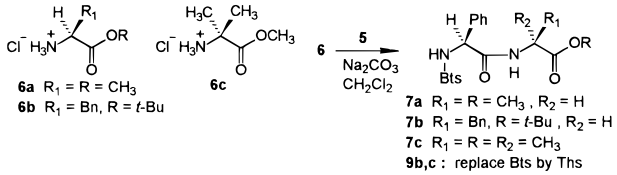
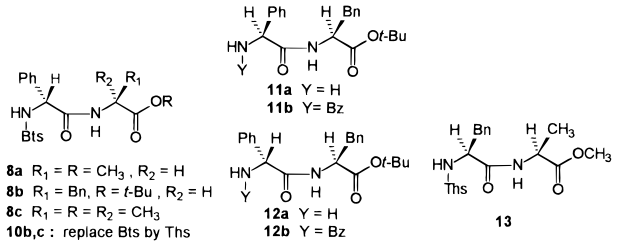
Several peptide coupling applications were explored in the sensitive Phg system. Recrystallized *N*-Bts-(*S*)-Phg was heated with 3 equiv of $SOCl_2$ in CH_2Cl_2, and the crude acid chloride **5** was reacted with amino esters **6** ($H_2O/Na_2CO_3-NaHCO_3/CH_2Cl_2$, 0−5 °C, 15 min). Protected dipeptides **7** were isolated



in high yield by crystallization (**7a**, 95%; **7b**, 89%; **7c**, 95% crude solid, 87% recrystallized), indicating essentially complete coupling despite the short reaction time. The minor diastereomers **8a** and **8b** and the enantiomer **8c** were prepared independently to establish detection limits in the crude products (**7a**, CH_3O ^{13}C satellite vs **8a** ^1H CH_3O signal, <0.1% **8a** detectable;[12] **7b**, *t*-BuO ^{13}C satellite vs ^1H *t*-BuO signal, 0.1% detectable;[12] **7c**, <0.1% **8c** detectable, CSP HPLC), as well as assay error (**7a**/**8a**, ±0.1%; **7b**/**8b**, ±0.2% crude, ±0.1% after crystallization; **7c**/**8c**, ±0.05%). No equally sensitive assay for *N*-Bts-Phg was found, thus the overall results were evaluated by comparing ee of the commercial starting materials (CSP HPLC: (*S*)-Phg, >99.9% ee; (*R*)-Phg, 99.2% ee; **6a**, 99.6% ee; **6b** ≥99.8% ee) with the de of **7a,b**/**8a,b** or the ee of **7c**/**8c**.



Diastereomer purity in *crude* **7a** (99.7% de) and **7b** (99.6% de) and enantiomeric purity in *crude* **7c** (99.8% ee) was consistent with a maximum of 0.1% racemization of the Phg subunit (≥99.8% retention) after allowing for experimental error and the purity of commercial reagents. Carpino *et al*. have shown that Cbz-Phg-F reacts with Aib-OCH_3 or with proline amide to give the dipeptides Cbz-Phg-Aib-OCH_3 (<1% racemization) and Cbz-Phg-Pro-NH_2 (< 0.1% diastereomer formation).[3a] The results with *N*-Bts-Phg-Cl are satisfactory in view of the simple reagents, methods, and greater reactivity compared to Cbz-Phg-F.[13]

Crystallization of the protected dipeptide **7b** improved ^{13}C satellite assay precision (99.8% de). Purified **7b** was deprotected by the slow addition of 50% H_3PO_2, refluxing THF−H_2O), and the resulting **11a** was converted into the more stable **11b** ($BzCl/NEt(i\text{-}Pr)_2/CH_2Cl_2$; 99% yield overall, 99.8% de; <0.1% diastereomer detection limit, ^{13}C satellite method). Using other reducing agents for deprotection, significant epimerization was detected in **11b** ($Na_2S_2O_4$/EtOH−H_2O, reflux, 2 h, 97% de; $NaHSO_3$, EtOH−H_2O, 6 h reflux, 88% de) in contrast to the H_3PO_2 results.

Similar reactions were briefly explored in the *N*-Ths-Phg series without optimizing yields. Thus, warming recrystallized *N*-Ths-(*S*)-Phg or *N*-Ths-(*R*)-Phg with thionyl chloride followed by reaction with **6c** (aqueous Na_2CO_3/CH_2Cl_2) produced **9c** or **10c** (64%). The enantiomers were distinguished by the chemical shift of the thisyl C−CH_3 signal in the presence of a chiral europium shift reagent (≥99.8% ee, ^{13}C satellite method; detection limit based on an authentic 99.7:0.3 mixture). Similarly, the Ths-Phg-Phe-OC$(CH_3)_3$ diastereomers **9b** and **10b** were obtained starting from **6b** and *N*-(*S*)-Ths-Phg and *N*-(*R*)-Ths-Phg, respectively (86−7% yield, ≥99.8% de). Deprotection of **9b** with Zn−HOAc followed by N-benzoylation afforded **11b** (92% overall, 99.8% de), and similar treatment of **10b** produced **12b** (≥99.7% de). Reaction of **9b** with $Na_2S_2O_4$/EtOH−H_2O (reflux) followed by benzoylation gave significant epimerization in **11b** (96% de). However, the standard H_3PO_2 deprotection of **9b** produced **11b** with 99.8% de (95% overall yield). Thus, the Bts and Ths protecting groups have very similar properties. The Bts group is cheaper, but the Ths group has small advantages in the rate of N-protection (aqueous suspension method), solubility, and crystallinity in some cases.

One additional dipeptide example was explored. Thus, (*S*)-phenylalanine (99.6% ee) was treated with ThsCl followed by thionyl chloride as usual. The resulting crude *N*-Ths-(*S*)-Phe-Cl was reacted with **6a** (99.6% ee) to give **13** in 70−75% overall yield ($Na_2CO_3-H_2O/CH_2Cl_2$ conditions). The thisyl C-CH_3 ^1H NMR signals of the diastereomers were resolved and 99.6% de was determined (^{13}C satellite method). However, if the crude *N*-Ths-(*S*)-Phe-Cl was recrystallized, then coupling with **6a** as before gave **13** with >99.8% de.

In summary, the Bts and Ths groups allow conversion of phenylglycine to the protected acid chloride **5**. Rapid coupling occurs with **6a**−**c** in the absence of additives, and the demanding Aib-OMe substrate affords **7c** with 99.8% ee. Deprotection of diastereomers **7b**/**8b** and **9b**/**10b** is possible without change in de using the inexpensive Zn/HOAc−EtOH or 50% H_3PO_2. The latter procedure involves homogeneous (moderately acidic) reducing conditions. We could find no prior use of H_3PO_2[14] in peptide synthesis or in N-deprotection. Other Bts- or Ths-protected amines are easily made and deprotected,[15,16] but these were not studied in detail since many protecting group options are available.[17] On the other hand, there are few alternatives that allow preparation of stable amino acid chlorides. The Bts-protected amino acid chlorides are recommended as practical reagents for difficult solution phase peptide-coupling reactions or for other applications where the high reactivity of an acid chloride is important.

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Supporting Information Available: Full experimental procedures, characterization, and assay data (24 pages). See any current masthead page for ordering and Internet access instructions.

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(11) H_3N-(+)-Trp-Met-Asp-Phe-NH_2 Cl-(−) (Sigma) was protected using excess BtsCl/NaOH (ca. 90% isolated). Cleavage with 50% H_3PO_2/DMF at rt followed by precipitation from dilute HCl gave ca. 70% recovery of material having the same NMR signals as the commercial peptide and minor signals due to a Bts-containing impurity.

(12) Dewey, R. S.; Schoenewaldt, E. F.; Joshua, H.; Paleveda, W. J., Jr.; Schwam, H.; Barkemeyer, H.; Arison, B. H.; Veber, D. F.; Denkewalter, R. G.; Hirschman, R. *J. Am. Chem. Soc.* **1968**, *90*, 3254. Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77.

(13) In a competition experiment, Aib-OCH_3·HCl/5% $NaHCO_3$ was added to 3 equiv of Cbz-Phg-F[3a] and 3 equiv of Bts-Phg-Cl in CH_2Cl_2. A chloride/fluoride reactivity advantage of 99:1 was established by NMR.

(14) Popik, V. V. In *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; Wiley: New York, 1995; Vol. 4, pp 2790.

(15) Glutathione and cysteine are known to cleave unsubstituted heteroarenesulfonamides derived from **1** and **2** by a nucleophilic mechanism: Colucci, D. F.; Buyske, D. A. *Biochem. Pharmacol.* **1965**, *14*, 457. Conroy, C. W.; Schwam, H.; Maren, T. H. *Drug Metab. Disp.* **1984**, *12*, 614. We thank a referee for these citations and for other valuable comments.

(16) Glycine (82% for thisyl protection, two-phase conditions; 89% for deprotection, Zn/HOAc−EtOH); indole (88% yield for protection using NaH + BtsCl in THF; 91% yield for deprotection with H_3PO_2 at rt, 2 h); trans-2-methyl-3-(triphenylmethyl)aziridine[5c] (77% for thisyl protection; 75% for deprotection, Zn/HOAc−EtOH); attempts to protect 2-phenylaziridine gave ring cleavage products; dibenzylamine (99% yield for thisyl protection, 94−5% for deprotection with Zn/HOAc−EtOH or Al−Hg/H_2O−THF).

(17) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd ed.; John Wiley & Sons, Inc.: New York, 1991.