2-Trifluoroacetylthiopyridine–1-Hydroxybenzotriazole: A Powerful Reagent for Peptide Synthesis

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A mixture of trifluoroacetylthiopyridine, the sodium salt of 1-hydroxybenzotriazole, and an acylamino acid is able to acylate amino acid esters in high yields with minimal racemization and should, therefore, be useful for fragment condensation reactions in the liquid phase.

Since the pioneering work by Geiger and König[1] on peptide synthesis using carbodiimide/1-hydroxybenzotriazole (HOBt), numerous reports have been published in which other condensing agents with HOBt as an additive (*e.g.* HOBt/BOP-Cl)[2] have been recommended or new reagents containing HOBt have been described.[3–7] Of the latter, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)[3] has long been recognised as one of the best coupling reagents. Recently, Swiss authors[6] have described the use of the corresponding urononium salts which have proved to be stable reagents for peptide synthesis and to give rise to minimal degrees of racemization. Peptide formation with these phosphonium and uronium salts can also be improved by the addition of HOBt.[6,8]

We have now found that a mixture of 2-trifluoroacetylthiopyridine with the sodium salt of HOBt (NaOBt) (**A**) is a further, highly effective coupling reagent. After addition of the acylamino acid to this mixture and an activation period of a few minutes between −20 °C and room temperature, the amino component is added and the mixture is allowed to warm to room temperature over a period of 10 h. The peptide can then be isolated in 80–85% yield with a racemization of between 0 and 1%.

In the formation of Z-Gly-Phe-Val-OMe (Z = benzyloxycarbonyl) (Table 1) in the absence of HOBt, extensive racemization takes place (86% d.e.) (run 1) while in the presence of HOBt practically no racemization occurs (run 2). The Hünig base is not so efficient as *N*-methylmorpholine (NMM) (run 3). No racemization can be detected by HPLC when NaOBt is used at −20 °C (run 4) and is still very low at room temperature (99% d.e.; run 5). Longer activation in tetrahydrofuran (THF) at room temperature gives rise to more extensive racemization (90% d.e.; run 6) whereas in CH_2Cl_2 under the same conditions only slight racemization is observed (98.5% d.e.; run 7). Furthermore, the reaction product is formed in 87% e.e. in the extremely sensitive activation of benzoylleucine using THF as solvent (Young test;[9] run 8). In CH_2Cl_2 the coupling proceeded to give the product in 98% e.e., an outcome which has never been achieved with any other coupling procedure (run 9). Coupling

Table 1 Coupling reactions with the trifluoroacetyl reagents **A–C**

Run	Product	Reagent	Base (1.0 equiv.)	Additive[f] (1.2 equiv.)	Activation time/min	Activation temp./°C	Solvent	Yield (%)	D.e. (%)[a]
1	Z-Gly-Phe-Val-OMe (Anteunis test[2])	**A**	NMM	—	10	−10	THF	85	86
2		**A**	NMM	HOBt	10	−10	THF	85	99
3		**A**	Hünig base[e]	HOBt	15	−10	THF	73	99
4[g]		**A**	—	NaOBt	15	−20	THF	82	100
5		**A**	—	NaOBt	3	RT[h]	THF	80	99
6		**A**	—	NaOBt	15	RT	THF	85	90
7		**A**	—	NaOBt	15	RT	CH_2Cl_2	80	98.5
8	Bz-Leu-Gly-OEt	**A**	—	NaOBt	10	−20	THF	85	E.e. = 87[b]
9	(Young test[9])	**A**	—	NaOBt	10	−20	CH_2Cl_2	75	E.e. = 98[c]
10	Z-Phe-MeLeu-OMe	**A**	—	NaOBt	20	−20	THF	60	99.5
11		**B**	—	NaOBt	10	−20	CH_2Cl_2	50	99.7
12	Z-Me-Leu-Val-OMe	**A**	—	NaOBt	20	−20	THF	80	99
13	Z-Gly-Phe-Val-OMe	**C**	NMM[d]	—	15	0	THF	37	99
14		**C**	NMM	HOBt	20	RT	THF	70	99

[a] Diastereoisomeric excess (d.e.) was determined by HPLC (^{1}H NMR spectroscopy in run 12). [b] Enantiomeric excess (e.e.) was determined from $[\alpha]_D^{20}$ −29.5 (*c* 1.20, EtOH); m.p. 153–154 °C. [c] E.e. was determined from $[\alpha]_D^{20}$ −33.3 (c 2.08, EtOH); m.p. 156–157 °C. [d] NMM = *N*-methylmorpholine. [e] Hünig base = *N*-ethyldiisopropylamine. [f] HOBt and NaOBt = 1-hydroxybenzotriazole and its sodium salt. [g] Run 4: to a stirred suspension of 180 mg (0.5 mmol) of Z-Gly-PheOH and 95 mg (0.6 mmol) of the sodium salt of 1-hydroxybenzotriazole in 2 ml of anhydrous THF were added 75 μl of 2-trifluoroacetylthiopyridine at −20 °C. The mixture was stirred for 20 min at −20 °C and treated with 90 mg (0.7 mmol) of HVal-OMe in 1 ml of anhydrous THF. Stirring at −20 °C was continued for 1 h and at room temp. for 1 h. The reaction mixture was diluted with *tert*-butyl methyl ether (40 ml), washed with 0.5 mol l^{-1} H_2SO_4 (5 ml), 1 mol l^{-1} NaOH (5 ml), and brine (5 ml), then dried ($MgSO_4$), and concentrated under reduced pressure. The degree of racemization was determined by HPLC on Merck LiChrosorb Si 60 (250-4) 7μ: t_R (Z-Gly-L-Phe-L-Val-OMe) = 3.27 min, 100%; t_R (Z-Gly-D-Phe-L-Val-OMe) = 4.50 min, 0%. The crude product was purified by column chromatography on silica gel with the eluents (*a*) hexane–ethyl acetate (1 : 1), and (*b*) ethyl acetate. Yield of pure (NMR, HPLC) tripeptide: 190 mg (82%). [h] RT = room temp.

of *N*-methylamino acid esters also takes place practically without racemization, albeit in yields of only 60% (run 10) whereas, in contrast, good yields are attained in couplings with *N*-methylleucine (run 12). Although esters of 3-cyano-4,6-dimethylpyridine-2-thiol are well suited for the activation of *N*-methylamino acids and couplings with *N*-methylamino acid derivatives,† use of the corresponding trifluoroacetate (**B**) does not provide any advantages (run 11). Finally we have also examined the use of 1-trifluoroacetoxybenzotriazole (**C**). The coupling reaction is very slow (run 13) and even the addition of HOBt (run 14) does not bring any improvement in comparison to 2-trifluoroacetylthiopyridine (**A**).

The trifluoroacetates used here were prepared by reactions of the corresponding, anhydrous sodium compounds with trifluoroacetic anhydride. For the preparation of 2-trifluoroacetylthiopyridine **A**, trifluoroacetic anhydride (TFAA; 7.7 ml, 55 mmol) was added to a solution of 6.84 g (51 mmol) of the sodium salt of 2-mercaptopyridine in CH_2Cl_2 (100 ml) during 15 min at room temperature and the mixture was then heated under reflux for 1 h. The resultant solution was filtered, the filtrate was evaporated, and the residue subjected to bulb-to-bulb distillation to furnish compound **A**, yield: 8.6 g (80%), b.p. 50–60 °C at 0.1 mbar.

4,6-Dimethyl-5-cyano-2-trifluoroacetylthiopyridine **B** was obtained similarly in 73% yield, b.p. 100 °C at 10^{-3} mbar. 1-Trifluoroacetoxybenzotriazole **C** was obtained in 60% yield. This product may explode on heating and should, therefore, not be distilled but rather purified by recrystallization from hexane–dichloromethane, m.p. 79–81 °C.

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† Yields of 80–90%, racemization <0.5%. Using this method, which has never been cited, Z-(*R*)-Ala-MeLeu-MeLeu-MeVal-OH, the critical tetrapeptide of cyclosporin, was prepared free of racemization in 61% yield.[10]

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