# Sulfonate Esters of 1-Hydroxypyridin-2(1*H*)-one and Ethyl 2-Cyano-2-(hydroxyimino)acetate (Oxyma) as Effective Peptide Coupling Reagents to Replace 1-Hydroxybenzotriazole and 1-Hydroxy-7-azabenzotriazole

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**A new family of sulfonate ester-type coupling reagents is described which differs in its leaving group. The sulfonate ester coupling reagents ethyl 2-cyano-2-(naphthalen-2-ylsulfonyloxyimino)acetate (NpsOXY), and ethyl 2-cyano-2-(tosyloxyimino)acetate (TsOXY) are more efficient alternatives to the benzotriazole sulfonate esters in terms of racemization suppression and coupling effectiveness. Both oxyma and its related sulfonate esters can be handled with a considerably lower risk than the explosive benzotriazole and its derivatives. 2-Oxopyridin-1(2*H*)-yl naphthalene-2-sulfonate (NpsOPy) and 2-oxopyridin-1(2*H*)-yl 4-methylbenzenesulfonate (TsOPy) sulfonate esters derived from 1-hydroxypyridin-2(1*H*)-one were also successfully used as new coupling reagents requiring a longer preactivation time during the coupling process. An improvement in yield and almost comparable optical purity to that of the 1-hydroxy-benzotriazole (HOBt) and 1-hydroxy-7-azabenzotriazole (HOAt) analogues was observed.**

**Key words** oxyma; solution phase methodology; coupling reagent; peptide synthesis; pyridone; sulfonate ester

The activation of carboxylic acids for the formation of amides or esters is an important process usually carried out using the so-called peptide coupling reagents.[1—3] This is followed by the reaction with the amine moiety of another amino acid to produce the desired peptide. The effective formation of a peptide bond not only depends on the reaction rate and yield but also on the suppression of the undesired racemization and other side reactions and thus minimizes the loss of the optical integrity at the chiral center of the carboxylic component. Preventing the loss of configuration is one of the major challenges in peptide synthesis.[4—8]

The activation of carboxylic acids using phosphonium[9—13] and uranium salts[14—17] is a fast, reliable process. These salts are prepared from a phosphonium or uronium cation bonded generally to a XO-group, normally a hydroxylamine derivative. These salts combine the functions of activating agents with suppressing additives and were successfully introduced in peptide synthesis.[18] A common method of minimizing the loss of configuration during stepwise coupling in solution phase is to add an additive such as 1-hydroxy-benzotriazole (HOBt) to the coupling mixture.[19] Recently, 1-hydroxy-7-azabenzotriazole (HOAt) and its derived phophonium or uronium/guanidinuim salts have been described as favorable coupling additives or reagents for both solution[20] and solid-phase synthesis.[21] HOAt has been reported to be more efficient than HOBt because of an anchimeric assistance effect caused by the pyridine ring.[22,23] These compounds enhance coupling yields and reduce the loss of chiral integrity during stepwise coupling.

Esters of sulfonic acids and HOBt have been used for peptide coupling.[24—28] The reactivity of these sulfonate esters was shown to be directly related to the presence of electron-withdrawing substituents in both the HOBt and the sulfonic acid moieties.[29—34] This methodology has few applications, as the coupling process depends on the basicity and reactivity of the amino component. The use of such sulfonate esters for *in situ* coupling was often accompanied by the formation of a sulfonamide byproduct.[24,30] Such byproduct formation can be avoided by preactivation of the reactive carboxylic acid component.

On the other hand, the use of a preactivation step is counterproductive, since the loss of configuration at the C-terminal carboxylic acid residue directly increases with preactivation time.[35] The replacement of HOBt by HOAt was expected to reduce the extent of configurational loss, but, on the contrary, the advantages of HOAt were lost during long preactivation times.[33,34]

Oxyma (ethyl 2-cyano-2-(hydroxyimino)acetate) **4** has been tested as an additive for use in the carbodiimide approach for the formation of peptide bonds. It showed better performance than the classical HOBt and HOAt, which have recently been reported to exhibit explosive properties. Oxyma displayed a remarkable capacity to inhibit racemization, together with impressive coupling efficiency, in both automated and manual synthesis, superior to those of HOBt and at least comparable to those of HOAt.[36—38]

Stability assays showed that there was no risk of capping the resin under standard coupling conditions. Oxyma can be handled with considerably lower risk than the explosive benzotriazole, as determined by calorimetric techniques.[36,37]

In the hope of suppressing the loss of chiral integrity during stepwise coupling and enhancing the coupling yield, 1-hydroxypyridin-2(1*H*)-one **3** and ethyl 2-cyano-2-(hydroxyimino)acetate **4** (oxyma) are used to replace HOBt **1** and HOAt **2**. The reactivity of the sulphonate esters is expected to be directly related to the stability of the leaving group anions ($OBt^-$, $OAt^-$, $PyO^-$ and $CNOEtO^-$), and accordingly related to their p$K_a$ values.[39,40] The p$K_a$ values of the additives HOBt **1** and HOAt **2**, 1-hydroxypyridin-2(1*H*)-one **3**, and ethyl 2-cyano-2-(hydroxyimino)acetate **4** are 4.60, 3.47,[41,42] 4.82,[43] and 4.60,[44] respectively.

Based on the p$K_a$ values of the additives, the sulphonate esters of HOAt are expected to be the most reactive coupling reagents. Ethyl 2-cyano-2-(hydroxyimino)acetate **4** and HOBt **1** sulphonate esters are probably similar in reactivity as coupling reagents. The least reactive coupling reagents should be

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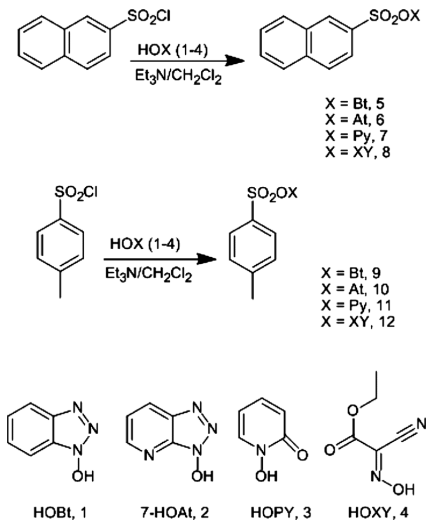


Chart 1. Synthesis of the Sulfonate Ester-Type Coupling Reagents

those containing the 1-hydroxypyridin-2(1*H*)-one **3** moiety.

The 2-naphthalenesulfonyl esters **5**—**8** and 4-tosyl esters **9**—**12** were prepared *via* the reaction of 2-naphthalenesulfonyl chloride or 4-tosyl chloride with HOBt **1**, HOAt **2**, 1-hydroxypyridin-2(1*H*)-one **3**, or ethyl 2-cyano-2-(hydroxyimino)acetate **4** in the presence of $Et_3N$ in anhydrous $CH_2Cl_2$ under a nitrogen atmosphere at 0 °C (Chart 1). Recrystallization from $CH_2Cl_2$–hexane gave pure reagents as colorless crystals. These reagents are suitable for long-term storage and are stable toward moisture in the air. Elemental analysis and NMR spectroscopy confirmed the structure of compounds **5**—**12**.

These coupling reagents **5**—**12** were examined in the stepwise coupling of four previously studied model systems,[22] Z-Val-Val-$OCH_3$ **13**, Z-Val-Ala-$OCH_3$ **14**, Z-Phe-Val-$OCH_3$ **15**, and Z-Phe-Ala-$OCH_3$ **16** to test for configurational control. The results of the different coupling reagents are given in Tables 1—4.

The extent of racemization during the preparation of the model systems was monitored using proton NMR analysis. The $OCH_3$ units of esters **13**, **14**, **15**, and **16** ($\delta$ 3.73, 3.74, 3.68, and 3.70, respectively) were monitored. These correspond to the LL enantiomers. The slightly downshielded peaks in the range of $\delta$ 3.69—3.87 correspond to the DL enantiomers. The assignment of the peaks was confirmed by the authentic syntheses of the four dipeptide models (Table 5). The coupling efficiency of the different coupling reagents was investigated for models **13**—**16** at various preactivation times in the presence and absence of an additive. All coupling reactions were performed in the presence of 2 equivalents of *N*,*N*-diisopropylethylamine (DIEA) as a base and in *N*,*N*-dimethylformamide (DMF) as a solvent.

The preparation of the dipeptide model Z-Val-Val-$OCH_3$ **13** using 3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl naphthalene-2-sulfonate (NpsOAt, **6**) as a coupling reagent after 2 h preactivation of Z-Val-OH (entry 1, Table 1) provided a good yield and the loss of configuration was small (1.6%). Preactivation of Z-Val-OH for 30 min (entry 2, Table 1) or for 3 min (entry 3, Table 1) in the latter case in the presence of 1 eq of HOAt as an additive yielded similar results. Both coupling reactions gave a good yield with the loss of configuration of

Table 1. Effect of Coupling Reagent and Preactivation Time on the Extent of Inversion at the First Valine during Formation of Z-Val-Val-OMe **13** in DMF (Solution Phase Synthesis)[a]

| Entry | Coupling reagent | Preactivation time (min) | Yield (%) | DL (%)[b] |
|---|---|---|---|---|
| 1 | NpsOAt | 120 | 91.5 | 1.6 |
| 2 | NpsOAt/HOAt | 30 | 90.7 | 1.8 |
| 3 | NpsOAt/HOAt | 3 | 92.1 | 0.9 |
| 4 | NpsOBt | 120 | 88.8 | 1.1 |
| 5 | NpsOBt/HOBt | 30 | 90.5 | 1.6 |
| 6 | NpsOBt/HOBt | 3 | 90.4 | 1.5 |
| 7 | NpsOPy | 120 | 84.5 | 1.9 |
| 8 | NpsOPy/HOPy | 30 | 80.9 | 8.7 |
| 9 | NpsOPy/HOPy | 3 | 75.7 | 3.2 |
| 10 | NpsOXY | 30 | 66.6 | 2.5 |
| 11 | NpsOXY/HOXY | 3 | 92.5 | 1.1 |
| 12 | TsOAt | 120 | 89.7 | 1.0 |
| 13 | TsOAt/HOAt | 30 | 90.3 | 1.6 |
| 14 | TsOBt | 120 | 79.7 | 3.7 |
| 15 | TsOBt/HOBt | 30 | 85.9 | 1.8 |
| 16 | TsOPy | 120 | 75.8 | 18.4 |
| 17 | TsOPy/HOPy | 30 | 59.74 | 0.5 |
| 18 | TsOXY/HOXY | 30 | 88.5 | 3.3 |
| 19 | TsOXY/HOXY | 3 | 92.6 | 1.2 |

*a*) Couplings were carried out with 2 eq DIEA, following the procedure mentioned in Experimental. *b*) $^1$H-NMR spectra was used to determine the DL ratio. The OMe group was observed at the range of 3.72—3.73 ppm for the LL enantiomers and at 3.75—3.87 ppm for the DL enantiomers.

Table 2. Effect of Coupling Reagent and Preactivation Time on the Extent of Inversion at Valine during Formation of Z-Val-Ala-OMe **14** in DMF (Solution Phase Synthesis)[a]

| Entry | Coupling reagent | Preactivation time (min) | Yield (%) | DL (%)[b] |
|---|---|---|---|---|
| 1 | NpsOAt | 120 | 93.6 | 1.3 |
| 2 | NpsOBt | 120 | 92.5 | 2.8 |
| 3 | NpsOBt/HOBt | 30 | 91.9 | 1.7 |
| 4 | NpsOPy | 120 | 69.2 | 2.5 |
| 5 | NpsOPy/HOPy | 30 | 67.4 | 5.7 |
| 6 | NpsOXY | 30 | 78.5 | 1.7 |
| 7 | NpsOXY | 3 | 90.7 | 2.8 |
| 8 | TsOAt | 120 | 90.3 | 0.5 |
| 9 | TsOAt/HOAt | 30 | 92.9 | 1.6 |
| 10 | TsOBt | 120 | 84.4 | 3.6 |
| 11 | TsOPy | 120 | 69.5 | 5.1 |
| 12 | TsOPy/HOPy | 30 | 47.7 | 0.1 |
| 13 | TsOPy/HOPy | 120 | 48.4 | 2.9 |
| 14 | TsOXY | 30 | 25.3 | 3.1 |
| 15 | TsOXY | 3 | 91.6 | 1.4 |

*a*) Couplings were carried out with 2 eq DIEA, following the procedure mentioned in Experimental. *b*) $^1$H-NMR spectra was used to determine the DL ratio. The OMe group was observed at the range of 3.74—3.744 ppm for the LL enantiomers and at 3.76—3.79 ppm for the DL enantiomers.

1.8% and 0.9%, respectively. The purity of the dipeptide was further confirmed using HPLC, affording 98.02% purity. Further study of the use of NpsOAt as a coupling reagent was accomplished by preparing the dipeptides **14**, **15**, and **16**. Performing 2 h preactivation during the preparation of the dipeptide **14** provided very good yield (entry 1, Table 2), while the preparation of the dipeptides **15** and **16** under the same conditions afforded lower yields (entry 1, Table 3; entry 1, Table 4). Using the NpsOAt coupling reagent in the presence of HOAt as an additive after 30 min preactivation (entry 2, Table 4) improved the yield of the dipeptide **16**. While a decrease in optical purity from 2.0 to 4.9% was ob-

Table 3. Effect of Coupling Reagent and Preactivation Time on the Extent of Inversion at Phenylalanine during Formation of Z-Phe-Val-OMe **15** in DMF (Solution Phase Synthesis)[a)]

| Entry | Coupling reagent | Preactivation time (min) | Yield (%) | DL (%)[b)] |
|---|---|---|---|---|
| 1 | NpsOAt | 120 | 54.5 | 6.7 |
| 2 | NpsOBt | 120 | 69.4 | 12.9 |
| 3 | NpsOPy | 120 | 69.8 | 7.2 |
| 4 | NpsOXY | 3 | 92.5 | 4.8 |
| 5 | TsOAt | 120 | 45.8 | 11.0 |
| 6 | TsOAt/HOAt | 30 | 79.7 | 3.9 |
| 7 | TsOBt | 120 | 52.2 | 1.4 |
| 8 | TsOBt/HOBt | 30 | 89.8 | 2.9 |
| 9 | TsOPy | 120 | 63.7 | 4.1 |
| 10 | TsOXY | 3 | 91.5 | 2.0 |

*a*) Couplings were carried out with 2 eq DIEA, following the procedure mentioned in Experimental. *b*) $^1$H-NMR spectra was used to determine the DL ratio. The OMe group was observed at the range of 3.59—3.68 ppm for the LL enantiomers and at 3.69—3.82 ppm for the DL enantiomers.

Table 4. Effect of Coupling Reagent and Preactivation Time on the Extent of Inversion at Phenylalanine during Formation of Z-Phe-Ala-OMe **16** in DMF (Solution Phase Synthesis)[a)]

| Entry | Coupling reagent | Preactivation time (min) | Yield (%) | DL (%)[b)] |
|---|---|---|---|---|
| 1 | NpsOAt | 120 | 29.5 | 2.0 |
| 2 | NpsOAt/HOAt | 30 | 69.7 | 4.9 |
| 3 | NpsOBt | 120 | 56.5 | 0.5 |
| 4 | NpsOPy | 120 | 77.1 | 1.9 |
| 5 | NpsOPy/HOPy | 30 | 71.8 | 3.0 |
| 6 | NpsOXY | 3 | 89.8 | 1.1 |
| 7 | TsOAt | 120 | 41.2 | 5.0 |
| 8 | TsOAt/HOAt | 30 | 75.5 | 3.7 |
| 9 | TsOBt | 120 | 53.5 | 4.8 |
| 10 | TsOPy | 120 | 55.6 | 26.8 |
| 11 | TsOPy/HOPy | 30 | 39.2 | 0.5 |
| 12 | TsOXY | 3 | 92.5 | 2.4 |

*a*) Couplings were carried out with 2 eq DIEA, following the procedure mentioned in Experimental. *b*) $^1$H-NMR spectra was used to determine the DL ratio. The OMe group was observed at the range of 3.70—3.705 ppm for the LL enantiomers and at 3.72—3.76 ppm for the DL enantiomers.

served.

The yield and loss of configuration of the four dipeptide models **13**—**16** using 1*H*-benzo[*d*][1,2,3]triazol-1-yl naphthalene-2-sulfonate (NpsOBt, **5**) as a coupling reagent were similarly examined. The preparation of **13** after a preactivation time of 30 min (entry 5, Table 1) and 3 min (entry 6, Table 1) in the presence of HOBt as an additive gave similar yield percentages and loss of configuration compared with entry 4, Table 1, after 2 h preactivation time in the absence of the additive. The purity of the dipeptide **13** was 94.64%, which is slightly lower than that of the same dipeptide when using NpsOAt as a coupling reagent. Similar results were obtained when preparing the dipeptide **14**, with improvement in the optical purity of the dipeptide at shorter preactivation time and in the presence of an additive (entry 3, Table 2). Similarly, the dipeptides **15**, and **16** were prepared.

Comparing the coupling reactions when using NpsOAt and NpsOBt as coupling reagents in the preparation of dipeptides **13** and **14**, the yield and loss of configuration at different preactivation times, and in the presence or absence of an additive, were similar. When preparing models **15** and **16**, in which lower yield percentages compared with the latter models were found, using NpsOBt as a coupling reagent is better than using NpsOAt in terms of yield percentage, as the advantage of NpsOAt is lost during long preactivation times. In addition, the presence of an additive in the reaction medium results in a major improvement in the yield.

1-Hydroxypyridin-2(1*H*)-one (Py-OH) **3** has a larger p$K_a$ value (4.82) than HOBt (4.6) and HOAt (3.47) and accordingly forms less stable anion and its active ester is less reactive. This could be an advantage when using longer preactivation times. That is why it was of interest to use 2-oxopyridin-1(2*H*)-yl naphthalene-2-sulfonate (NpsOPy, **7**) as coupling reagent. The preparation of the dipeptide model **13** using NpsOPy **7** as coupling reagent was performed at different preactivation times and in the presence and absence of PyOH as an additive. It was observed that the longer preactivation time (2 h), (entry 7, Table 1), gave the highest yield percentage and optical purity. The purity of the dipeptide was confirmed using HPLC, affording 100% purity. This was further confirmed by preparing the dipeptide **14**, which also showed better yield and optical purity after 2 h preactivation in the absence of an additive (entry 4, Table 2). The dipeptides **15** and **16** were also prepared using the latter coupling reagent after 2 h preactivation. An improvement in the yield and optical purity of the dipeptides was observed (entry 3, Table 3; entry 4, Table 4).

The use of ethyl 2-cyano-2-(naphthalen-2-ylsulfonyloxyimino)acetate (NpsOXY, **8**) as a coupling reagent showed remarkable results. The coupling reaction for the preparation of the dipeptide model **13** was studied at different preactivation times. With increased preactivation time, the yield of the dipeptide decreased. The color of the preactivated solution at the beginning was yellow. If the reaction mixture is left for longer preactivation times, a change in color was observed, until it became very dark red. The coupling reaction affords a very good yield of the dipeptide **13** with 3 min preactivation in the presence of an additive (entry 11, Table 1). Allowing a longer preactivation time resulted in a decrease in the yield (entry 10, Table 1). The purity of the dipeptide was further confirmed using HPLC, affording 97.69% purity (Fig. 1). The dipeptides **14**, **15**, and **16** were prepared using the coupling reagent **8** after 3 min preactivation in the absence of an additive, giving a very good yield. NpsOXY **8** as a coupling reagent has the advantage over the other sulfonate esters studied of requiring a shorter preactivation time. In addition, all four dipeptide models studied were prepared in excellent yields.

Furthermore, 3*H*-[1,2,3]triazolo[4,5-*b*]pyridin-3-yl 4-methylbenzenesulfonate (TsOAt, **10**) was used as coupling reagent. The preparation of the dipeptides **13** and **14** after 2 h preactivation in the absence of an additive (entry 12, Table 1; entry 8, Table 2), and after 30 min preactivation in the presence of HOAt as an additive (entry 13, Table 1; entry 9, Table 2) were studied. The yield and optical purity in both cases were almost identical. During the preparation of the dipeptides **15** (entries 5 and 6, Table 3) and **16** (entries 7 and 8, Table 4) an improvement in both the yield and optical purity was observed with 30 min preactivation in the presence of an additive.

The preparation of the dipeptides using 1*H*-benzo[*d*][1,2,3]triazol-1-yl-4-methylbenzenesulfonate (TsOBt, **9**) as a

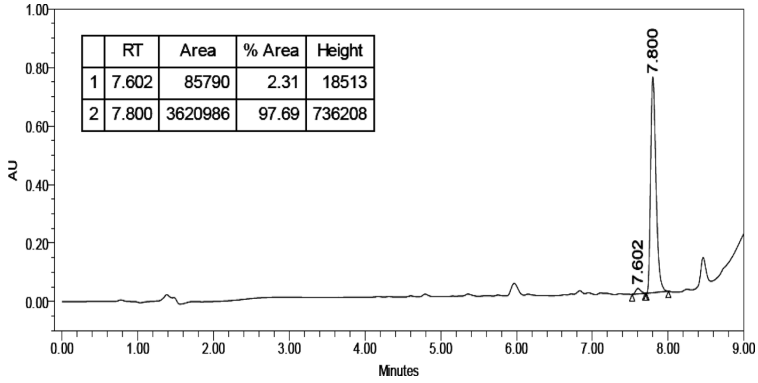


Fig. 1. The Purity of the Dipeptide **13** Was Confirmed by HPLC Analysis of the Crude Sample of Z-Val-Val-OMe

In this case NpsOXY is used as coupling reagent. Conditions: a Sunfire $C_{18}$ 60 Å column (4.6×100 mm, 4 $\mu$m), linear gradient over 25 min of 10 to 90% $CH_3CN$ in $H_2O$/0.1% TFA, flow rate 1.0 ml/min.

coupling reagent after 30 min preactivation in the presence of HOBt as an additive afforded an increase in yield and optical purity compared with the coupling reaction after 2 h preactivation. The purity of the dipeptide **13** prepared under these conditions was further confirmed using HPLC, showing 87.22% purity.

When using the coupling reagent 2-oxopyridin-1(2*H*)-yl 4-methylbenzenesulfonate (TsOPy, **11**) for the synthesis of the four dipeptides **13**—**16**, the following observations were clear. The coupling reactions using the latter coupling reagent require 2 h preactivation to obtain a good yield (entry 16, Table 1; entry 11, Table 2; entry 9, Table 3; and entry 10, Table 4). With only 30 min preactivation in the presence of an additive (PyOH) the yield is decreased, while the optical purity was improved (entry 17, Table 1; entry 12, Table 2; and entry 11, Table 4). Trying to improve the yield of the dipeptide **14** by allowing 2 h preactivation in the presence of an additive (entry 13, Table 2) afforded a yield similar to that obtained after 30 min preactivation, accompanied by a decrease in the optical purity (entry 12, Table 2). The purity of the dipeptide **13** prepared in this case was further confirmed using HPLC, showing 84.46% purity.

Generally it was also noticed that the coupling reactions using NpsOPy as coupling reagent gave higher yield, better optical purity, and higher purity of the dipeptides than when using its tosylate analogue TsOPy.

Finally, using ethyl 2-cyano-2-(tosyloxyimino)acetate (TsOXY, **12**) as a coupling reagent for the synthesis of the dipeptide **14** after 30 min preactivation in the absence of an additive afforded very low yield (entry 14, Table 2), while preparing the dipeptide under the same conditions after 3 min preactivation afforded a much better yield (91.6%), (entry 15, Table 2). The dipeptide **13** was prepared under the same conditions in the presence of the additive (ethyl 2-cyano-2-(hydroxyimino)acetate (HOXY)) affording good yield (entry 19, Table 1). Generally, better results were obtained with only 3 min preactivation in the presence or absence of an additive. The yield in all cases was greater than 90% and very low racemization was observed (entry 19, Table 1; entry 15, Table 2; entry 10, Table 3; entry 12, Table 4). The purity of the dipeptide **13** prepared in this case was further confirmed using HPLC, showing 97.07% purity.

The sulfonate ester coupling reagents are stable solids. Their stability was tested in DMF solution in an open vial exposed to the atmosphere. The stability of the reagents was examined with TLC (eluent, ethylacetate : hexane, 1 : 1) after 5, 24, and 48 h. No significant change was observed, which confirms the stability of the reagents in DMF solution, a solvent commonly used for peptide synthesis.

## Conclusion

NpsOXY and TsOXY were investigated as new coupling reagents. The coupling reactions were tested in the presence or in absence of oxyma as an additive. They showed clear superiority to their HOBt and HOAt analogues in terms of suppression of racemization and coupling efficiency in all four models prepared. They also had the advantage of requiring a short preactivation time. The oxyma sulfonate ester coupling reagents are more efficient alternatives to the benzotriazole sulfonate esters in terms of racemization suppression and coupling effectiveness. Both oxyma and its related sulfonate esters can be handled with a considerably lower risk than the explosive benzotriazole and its derivatives. NpsOPy and TsOPy sulfonate esters derived from 1-hydroxypyridin-2(1*H*)-one were also successfully used as new coupling reagents requiring longer preactivation time during the coupling process. The use of 1-hydroxypyridin-2(1*H*)-one as an additive during the coupling process helped to decrease the preactivation time. This was also accompanied by suppression of racemization and a decrease in yield. Generally, the naphthyl-derived coupling reagents are superior to the tosyl ones. In addition, using NpsOBt as a coupling reagent is advantageous over NpsOAt in terms of yield, as the advantage of NpsOAt is lost during long preactivation times. The presence of an additive in the reaction medium results in a marked improvement in yield and optical purity. Testing the stability of the sulfonate ester coupling reagents confirmed their stability in air.

### Experimental

Oxyma (ethyl 2-cyano-2-(hydroxyimino)acetate) and pyridone (1-hydroxypyridin-2(1*H*)-one) were obtained from commercial sources (Aldrich). DMF was of peptide-grade purity. All peptides were identified with $^1$H-NMR. The purity of the dipeptides was confirmed by HPLC analysis of crude samples. Conditions: a Sunfire $C_{18}$ 60-Å column (4.6×100 mm, 4 $\mu$m); linear gradient over 25 min of 10 to 90% $CH_3CN$ in $H_2O$/0.1% TFA; and flow rate 1.0 ml/min. TLC was performed on silica gel-protected aluminum sheets (type 60 GF254, Merck), and the spots were detected by exposure to a UV lamp at $\lambda$ 254 nm for a few seconds. Melting points were obtained in open capillary tubes using a Mel-Temp apparatus and were uncorrected. Infrared

Table 5. The $^1$H-NMR Spectrums of the Four Dipeptides Studied

| Dipeptide | $^1$H-NMR (500 MHz, $CDCl_3$) |
|---|---|
| Z-Val-Val-$OCH_3$ | $\delta$ 0.87—0.96 (m, 9H, $3CH_3$), 0.99 (d, 3H, $CH_3$), 2.09—2.17 (m, 2H, 2CH), 3.73 (s, 3H, $CH_3$), 4.05 (t, 1H, CH), 4.53 (dd, 1H, CH), 5.11 (s, 2H, $CH_2$), 5.40 (br d, 1H, NH, $D_2O$ exchangeable), 6.39 (br d, 1H, NH, $D_2O$ exchangeable), 7.30—7.35 (m, 5H, Ar-H). |
| Z-Val-Ala-$OCH_3$ | $\delta$ 0.92, 0.97 (2d, 6H, $2CH_3$), 1.40 (d, 3H, $CH_3$), 2.09—2.14 (m, 1H, CH), 3.74 (s, 3H, $CH_3$), 4.01 (t, 1H, CH), 4.57 (t, 1H, CH), 5.10 (s, 2H, $CH_2$), 5.38 (br d, 1H, NH, $D_2O$ exchangeable), 6.37 (br d, 1H, NH, $D_2O$ exchangeable), 7.30—7.36 (m, 5H, Ar-H). |
| Z-Phe-Val-$OCH_3$ | $\delta$ 0.80, 0.83 (2d, 6H, $2CH_3$), 2.07—2.10 (m, 1H, CH), 3.04—3.09 (m, 2H, $CH_2$), 3.68 (s, 3H, $CH_3$), 4.43—4.45 (m, 2H, 2CH), 5.09 (s, 2H, $CH_2$), 5.36 (br d, 1H, NH, $D_2O$ exchangeable), 6.27 (br d, 1H, NH, $D_2O$ exchangeable), 7.20—7.36 (m, 10H, Ar-H). |
| Z-Phe-Ala-$OCH_3$ | $\delta$ 1.32 (d, 3H, $CH_3$), 3.02—3.14 (m, 2H, $CH_2$), 3.70 (s, 3H, $CH_3$), 4.42—4.51 (m, 2H, 2CH), 5.09 (s, 2H, $CH_2$), 5.32 (br d, 1H, NH, $D_2O$ exchangeable), 6.31 (br d, 1H, NH, $D_2O$ exchangeable), 7.26—7.33 (m, 10H, Ar-H). |

(IR) spectra were recorded on a Perkin-Elmer 1600 series Fourier transform instrument as KBr pellets. NMR spectra were recorded on a 500 MHz JEOL and on a 400 MHz Varian mercury spectrometer at room temperature. Tetramethylsilane (TMS) was used as a reference for all NMR spectra, with chemical shifts reported as $\delta$ units (parts per million, ppm) relative to TMS. Elemental analyses were performed on a Perkin-Elmer 2400 elemental analyzer, and the values found were within $\pm$0.3% of the theoretical values. The compounds were illustrated using Chem Draw Ultra version 11, Cambridge Soft Corporation.

**General Method for the Synthesis of Sulfonate Esters (5—12)** To a suspension of 5 mmol of HOBt, HOAt, 1-hydroxypyridin-2(1*H*)-one, or ethyl 2-cyano-2-(hydroxyimino)acetate in 30 ml of anhydrous $CH_2Cl_2$ was added 0.71 ml (5 mmol) of triethylamine with magnetic stirring. The resulting clear yellow solution was cooled in an ice bath under an atmosphere of $N_2$ and treated slowly with 1 eq of 4-tosyl chloride or 2-naphthalenesulfonyl chloride. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. After dilution with 30 ml of $CH_2Cl_2$, the organic phase was washed with water and saturated aqueous NaCl (30 ml) and dried over $Na_2SO_4$ anhydrous. After removal of the solvent with a rotary evaporator, the residue was recrystallized from $CH_2Cl_2$/hexane to give the sulfonate esters.

1*H*-Benzo[*d*][1,2,3]triazol-1-yl naphthalene-2-sulfonate (NpsOBt, **5**): This compound was obtained as colorless crystals, 1.33 g (82%), mp 127—128 °C; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 7.43 (t, 1H, Ar-H, $J$=8.4 Hz), 7.57 (t, 1H, Ar-H, $J$=7.7 Hz), 7.65 (t, 2H, Ar-H, $J$=8.4 Hz), 7.75 (t, 1H, Ar-H, $J$=8.4 Hz), 7.88—8.00 (m, 4H, Ar-H), 8.07 (d, 1H, Ar-H, $J$=8.4 Hz), 8.45 (s, 1H, Ar-H). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 109.57, 120.41, 123.16, 125.46, 128.31, 128.41, 128.85, 128.98, 129.45, 129.87, 130.41, 130.81, 131.95, 132.81, 136.46, 143.80. *Anal.* Calcd for $C_{16}H_{13}N_3O_3S$: C, 59.07; H, 3.41; N, 12.92. Found: C, 58.86; H, 3.26; N, 13.09.

3*H*-[1,2,3]Triazolo[4,5-*b*]pyridin-3-yl naphthalene-2-sulfonate (NpsOAt, **6**): This compound was obtained as colorless crystals, 1.48 g (91%), mp 142—143 °C (lit. mp 140—141 °C)[14]; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 7.43 (dd, 1H, Ar-H, $J$=8.4, 4.6 Hz), 7.67 (t, 1H, Ar-H, $J$=8.4 Hz), 7.75 (t, 1H, Ar-H, $J$=8.4 Hz), 7.96—7.99 (m, 3H, Ar-H), 8.08 (d, 1H, Ar-H, $J$=8.4 Hz), 8.36 (d, 1H, Ar-H, $J$=9.2 Hz), 8.54 (s, 1H, Ar-H), 8.71 (d, 1H, Ar-H, $J$=5.4 Hz). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 121.36, 123.30, 128.28, 128.36, 129.52, 129.57, 129.86, 130.33, 130.75, 131.97, 132.80, 134.53, 136.44, 152.45. *Anal.* Calcd for $C_{15}H_{10}N_3O_3S$: C, 55.21; H, 3.09; N, 17.17. Found: C, 55.43; H, 2.88; N, 16.92.

2-Oxopyridin-1(2*H*)-yl naphthalene-2-sulfonate (NpsOPy, **7**): This compound was obtained as colorless crystals, 1.32 g (88%), mp 93—94 °C; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 6.15 (dt, 1H, Ar-H, $J$=6.8, 2.0 Hz), 6.48 (dd, 1H, Ar-H, $J$=9.2, 2.0 Hz), 7.28 (dt, 1H, Ar-H, $J$=6.8, 2 Hz), 7.60—7.66 (m, 2H, Ar-H), 7.72 (dt, 1H, Ar-H, $J$=6.8, 1.2 Hz), 7.94—8.05 (m, 4H, Ar-H), 8.57 (s, 1H, Ar-H). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 105.44, 123.45, 123.77, 128.19, 128.42, 128.59, 129.87, 129.92, 130.48, 130.60, 130.63, 131.97, 132.48, 136.39, 137.10, 139.66, 157.08. *Anal.* Calcd for $C_{15}H_{11}NO_4S$: C, 59.79; H, 3.68; N, 4.65. Found: C, 59.85; H, 3.83; N, 4.54.

Ethyl 2-Cyano-2-(naphthalen-2-ylsulfonyloxyimino)acetate (NpsOXY, **8**): This compound was obtained as colorless crystals, 1.53 g (92%), mp 94—95 °C; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 1.35 (t, 3H, $CH_3$, $J$=7.7 Hz), 4.38 (quart, 2H, $CH_2$, $J$=7.7 Hz), 7.69, 7.75 (2t, 2H, Ar-H, $J$=7.7 Hz), 7.95—7.97 (m, 2H, Ar-H), 8.03 (d, 1H, Ar-H, $J$=2.3 Hz), 8.05 (d, 1H, Ar-H, $J$=3.8 Hz), 8.68 (s, 1H, Ar-H). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 13.98, 64.69, 106.19, 123.14, 128.22, 128.37, 129.84, 130.00, 130.09, 130.58, 131.46, 131.96, 132.35, 155.94. *Anal.* Calcd for $C_{15}H_{12}N_2O_5S$: C, 54.21; H, 3.64; N, 8.43. Found: C, 54.07; H, 3.79; N, 8.67.

1*H*-Benzo[*d*][1,2,3]triazol-1-yl 4-methylbenzenesulfonate (TsOBt,**9**): This compound was obtained as colorless crystals, 1.14 g (79%), mp 78—79 °C (lit. mp 80—81 °C)[14]; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 2.52 (s, 3H, $CH_3$), 7.38—7.44 (m, 3H, Ar-H), 7.57 (t, 1H, Ar-H, $J$=7.7 Hz), 7.62 (d, 1H, Ar-H, $J$=8.6 Hz), 7.76 (d, 2H, Ar-H, $J$=8.6 Hz), 7.99 (d, 1H, Ar-H, $J$=8.6 Hz). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 22.08, 109.61, 120.35, 125.39, 127.14, 128.83, 129.16, 129.37, 129.90, 130.60, 143.01, 148.12. *Anal.* Calcd for $C_{13}H_{11}N_3O_3S$: C, 53.97; H, 3.83; N, 14.52. Found: C, 54.16; H, 3.98; N, 14.74.

3*H*-[1,2,3]Triazolo[4,5-*b*]pyridin-3-yl 4-methylbenzenesulfonate (TsOAt, **10**): This compound was obtained as colorless crystals, 1.13 g (78%), mp 131—132 °C (lit. mp 133—134 °C)[14]; $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 2.51 (s, 3H, $CH_3$), 7.41—7.45 (m, 3H, Ar-H), 7.87 (d, 2H, Ar-H, $J$=7.6 Hz), 8.38 (d, 1H, Ar-H, $J$=8.4 Hz), 8.77 (dd, 1H, Ar-H, $J$=4.6, 1.6 Hz). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 22.13, 121.37, 129.53, 129.65, 129.98, 130.85, 134.55, 140.65, 148.05, 152.50. *Anal.* Calcd for $C_{12}H_{10}N_4O_3S$: C, 49.65; H, 3.47; N, 19.30. Found: C, 49.88; H, 3.54; N, 19.12.

2-Oxopyridin-1(2*H*)-yl 4-methylbenzenesulfonate (TsOPy, **11**): This compound was obtained as colorless crystals, 1.08 g (81%), mp 94—95 °C. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 2.47 (s, 3H, $CH_3$), 6.15 (dt, 1H, Ar-H, $J$=6.4, 1.6 Hz), 6.51 (dd, 1H, Ar-H, $J$=9.2, 1.6 Hz), 7.29 (m, 1H, Ar-H), 7.37 (d, 2H, Ar-H, $J$=8.0 Hz), 7.59 (dd, 1H, Ar-H, $J$=7.2, 2.0 Hz), 7.89 (d, 2H, Ar-H, $J$=8.4 Hz). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 22.19, 105.32, 123.41, 130.01, 130.07, 130.09, 130.14, 130.19, 130.67, 137.16, 139.60, 147.44, 157.04. *Anal.* Calcd for $C_{12}H_{11}NO_4S$: C, 54.33; H, 4.18; N, 5.28. Found: C, 54.15; H, 3.97; N, 5.45.

Ethyl 2-Cyano-2-(tosyloxyimino)acetate (TsOXY, **12**): This compound was obtained as colorless crystals, 1.27 g (86%), mp 65—66 °C. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$: 1.37 (t, 3H, $CH_3$, $J$=6.9 Hz), 2.48 (s, 3H, $CH_3$), 4.40 (quart, 2H, $CH_2$, $J$=6.9 Hz), 7.40 (d, 2H, Ar-H, $J$=7.6 Hz), 7.92 (d, 2H, Ar-H, $J$=7.6 Hz). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta$: 14.00, 22.00, 64.66, 106.20, 129.64, 130.18, 130.36, 131.26, 147.39, 156.02. *Anal.* Calcd for $C_{12}H_{12}N_2O_5S$: C, 48.64; H, 4.08; N, 9.45. Found: C, 48.89; H, 4.31; N, 9.68.

**General Method for the Synthesis of Dipeptides** To a solution of 0.1 mmol of Z-Val-OH or Z-Phe-OH and 0.2 mmol of DIEA in 2 ml of DMF was added 0.1 mmol of the appropriate coupling reagent. The reaction mixture was stirred for preactivation at different times depending on the conditions of the entry studied, followed by the addition of a solution of 0.1 mmol of Val-OMe·HCl or Ala-OMe·HCl and 0.1 mmol of DIEA in 1 ml of DMF. The reaction mixture was stirred overnight. After dilution with 50 ml of ethyl acetate, the organic phase was washed with 5% citric acid (3×20 ml), saturated $NaHCO_3$ (3×20 ml) and saturated NaCl (3×20 ml) and dried over $Na_2SO_4$ anhydrous. After removal of the solvent with a rotary evaporator, the residue was recrystallized from $CH_2Cl_2$/hexane to give the dipeptide (Table 5).

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**References and Notes**

1) Amino acids and peptides are abbreviated and designated following the rules of the IUPAC-IUB Commission of Biochemical Nomenclature [*J. Biol. Chem.*, **247**, 977 (1972)].
2) Albericio F., Carpino L. A., "Methods Enzymol., Solid-Phase Peptide Synthesis," Vol. 289, ed. by Fields G. B., Academic Press, Orlando, FL, 1997, pp. 104—126.
3) Albericio F., Kates S. A., "Solid-Phase Synthesis A Practical Guide," ed. by Kates S. A., Albericio F., Marcel Dekker, New York, 2000, pp.

275—330.
4) Montalbetti C. A. G. N., Falque V., *Tetrahedron*, **61**, 10827—10852 (2005).
5) Han S.-Y., Kim Y.-A., *Tetrahedron*, **60**, 2447—2467 (2004).
6) Kemp D. S., "Peptides Analysis Synthesis Biology," Vol. 1, ed. by Gross E., Meienhofer J., Academic Press, New York, 1979, p. 315.
7) Kovacs J., "Peptides Analysis Synthesis Biology," Vol. 2, ed. by Gross E., Meienhofer J., Academic Press, New York, 1979, p. 485.
8) Benoiton N. L., "Peptides Analysis Synthesis Biology," Vol. 5, ed. by Gross E., Meienhofer J., Academic Press, New York, 1983, p. 217.
9) Castro B., Dormoy J.-R., Evin G., Selve C., *Tetrahedron Lett.*, **16**, 1219—1222 (1975).
10) Castro B., Dormoy J.-R., Dourtoglou B., Evin G., Selve C., Ziegler J.-C., *Synthesis*, **1976**, 751—752 (1976).
11) Dormoy J.-R., Castro B., *Tetrahedron Lett.*, **20**, 3321—3322 (1979).
12) Le-Nguyen D., Heitz A., Castro B., *J. Chem. Soc. Perkin Trans. 1*, **28**, 1915—1919 (1987).
13) Coste J., Le-Nguyen D., Castro B., *Tetrahedron Lett.*, **31**, 205—208 (1990).
14) Dourtoglou B., Ziegler J.-C., Gross B., *Tetrahedron Lett.*, **19**, 1269—1272 (1978).
15) Dourtoglou B., Ziegler J.-C., Gross B., *Synthesis*, **1984**, 572 (1984).
16) Knorr K., Trzeciak A., Bannwarth W., Gillesseu D., *Tetrahedron Lett.*, **30**, 1927—1930 (1989).
17) El-Faham A., Khattab Sh. N., Abdul-Ghani M., Albericio F., *Eur. J. Org. Chem.*, **2006**, 1563—1573 (2006).
18) Williams A., Ibrahim I. T., *Chem. Rev.*, **81**, 589—636 (1981).
19) König W., Geiger R., *Chem. Ber.*, **103**, 788—798 (1970).
20) Carpino L. A., Ismail M., Truran G., Mansour E. M. E., Iguchi S., Ionescu D., El-Faham A., Riemer C., Warrass R., *J. Org. Chem.*, **64**, 4324—4338 (1999).
21) Carpino L. A., El-Faham A., Minor C., Albericio F., *J. Chem. Soc. Chem. Commun.*, **1994**, 201—203 (1994).
22) Carpino L. A., *J. Am. Chem. Soc.*, **115**, 4397—4398 (1993).
23) Carpino L. A., El-Faham A., *J. Org. Chem.*, **60**, 3561—3564 (1995).
24) Itoh M., Nojima H., Notani J., Hagiwara D., Takai K., *Tetrahedron Lett.*, **15**, 3089—3092 (1974).
25) Devedas B., Pandey R. K., Mathur K. B., *Ind. J. Chem.*, **16**, 1026 (1978).
26) Kundu B., Srivastava A., Devedas B., Mathur K. B., *Ind. J. Chem.*, **28B**, 604—605 (1989).
27) Kundu B., Shukla S., Shukla M., *Tetrahedron Lett.*, **35**, 9613—9614 (1994).
28) Khare S. K., Singh G., Agarwal K. C., Kundu B., *Protein Pept. Lett.*, **5**, 171—174 (1998).
29) Furukawa M., Hokama N., Okawara T., *Synthesis*, **1983**, 42—47 (1983).
30) Topuzzan N. O., Matirosyan M. S., *J. Org. Chem.* (USSR), **27**, 2148—2153 (1991).
31) Devedas B., Kundu B., Srivastava A., Mathur K. B., *Tetrahedron Lett.*, **34**, 6455—6458 (1993).
32) Kundu B., Argawal K. C., *J. Chem. Res., Synop.*, **1996**, 200 (1996).
33) Carpino L. A., El-Faham A., Albericio F., *Tetrahedron Lett.*, **35**, 2279—2282 (1994).
34) Han Y., Albericio F., Barany G., *J. Org. Chem.*, **62**, 4307—4312 (1997).
35) Carpino L. A., Xia J., Zhang C., El-Faham A., *J. Org. Chem.*, **69**, 62—71 (2004).
36) Subirós-Funosas R., Prohens R., Barbas R., El-Faham A., Albericio F., *Chem. Eur. J.*, **15**, 9394—9403 (2009).
37) El-Faham A., Subirós-Funosas R., Prohens R., Albericio F., *Chem. Eur. J.*, **15**, 9404—9416 (2009).
38) Subirós-Funosas R., Acosta G. A., El-Faham A., Albericio F., *Tetrahedron Lett.*, **50**, 6200—6202 (2009).
39) Khattab Sh. N., Hassan S. Y., Hamed E. A., El-Faham A., *J. Chem. Res.*, **April**, 247—251 (2007).
40) Khattab Sh. N., Hassan S. Y., Hamed E. A., Albericio F., El-Faham A., *Bull. Korean Chem. Soc.*, **31**, 75—81 (2010).
41) Carpino L. A., Imazumi H., Foxman B. M., Vela M. J., Henklein P., El-Faham A., Klose J., Bienert M., *Org. Lett.*, **15**, 2253—2256 (2000).
42) Koppel I., Koppel J., Leito I., Pihl V., Grehn L., Ragnarsson U., *J. Chem. Res. Synop.*, **1993**, 446 (1993).
43) Fathalla M. F., Khattab Sh. N., unpublished data.
44) Itoh M., *Bull. Chem. Soc. Jpn.*, **46**, 2219—2221 (1973).