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Synthesis and Application of *N*-Hydroxylamine Derivatives as Potential Replacements for HOBt

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Several heterocycles containing *N*-hydroxylamine were prepared and tested as coupling additives to replace the use of *N*-hydroxybenzotriazole (HOBt) derivatives. On the basis of our results on coupling yield and racemization-suppressing properties, we propose *N*-hydroxyindolin-2-one and 6-chloro-*N*-hydroxy-2-phenylbenzimidazole as suitable substitutes for HOBt.

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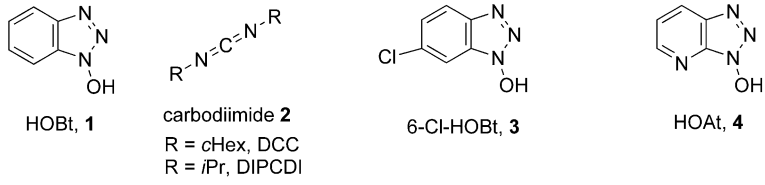
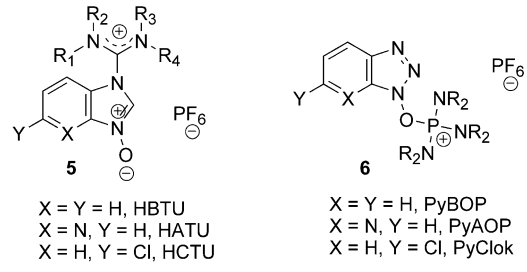
Introduction

For many years, 1-hydroxybenzotriazole (HOBt, **1**)[1] is present in almost all peptide couplings, especially as an additive in combination with carbodiimides (**2**).[1] Later, other benzotriazole derivatives, such as 6-chloro-1-hydroxybenzotriazole (6-Cl-HOBt, **3**)[2] and 1-hydroxy-7-azabenzotriazole (HOAt, **4**),[3] were introduced. The main role of HOBt derivatives is to react with *O*-acylisourea, formed by reaction of the protected amino acid, to reduce racemization associated with *O*-acylisourea and to prevent its rearrangement to the inactive *N*-acylurea.[4]

During the last decades, aminium/uronium and phosphonium derivatives of HOBt (**5**, **6**) have been proposed for the synthesis of peptides.[5] In the presence of base, these stand-alone coupling reagents react with the protected amino acids to render the corresponding oxybenzotriazole esters.[6]

One of the main drawbacks associated with the use of HOBt derivatives is their explosive properties, which are often not always properly referenced in the literature.[5,7] This explosive tendency is associated with the triazo system, which must be handled with care.[8] This risk is greater during the industrial production of peptides, which often involves the handling of tons of these derivatives.





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Results and Discussion

Here we describe the preparation of a number of *N*-hydroxylamine-containing heterocycles for tests as coupling additives. Both coupling yield and racemization-suppressing properties were studied.

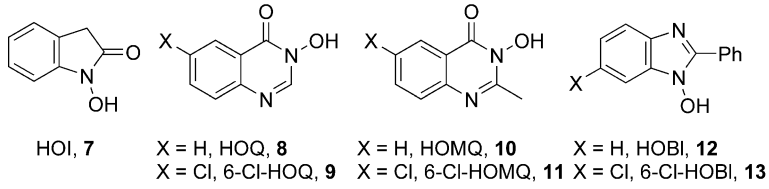
In addition to the presence of the hydroxylamine moiety, none of the derivatives prepared contained two or more nitrogen atoms in consecutive positions as occurs in hydroxybenzotriazole derivatives. Furthermore, some extra Cl atoms were introduced in an attempt to mimic the positive effect shown by Cl in the case of the 6-Cl-HOBt vs. HOBt.[2,9]

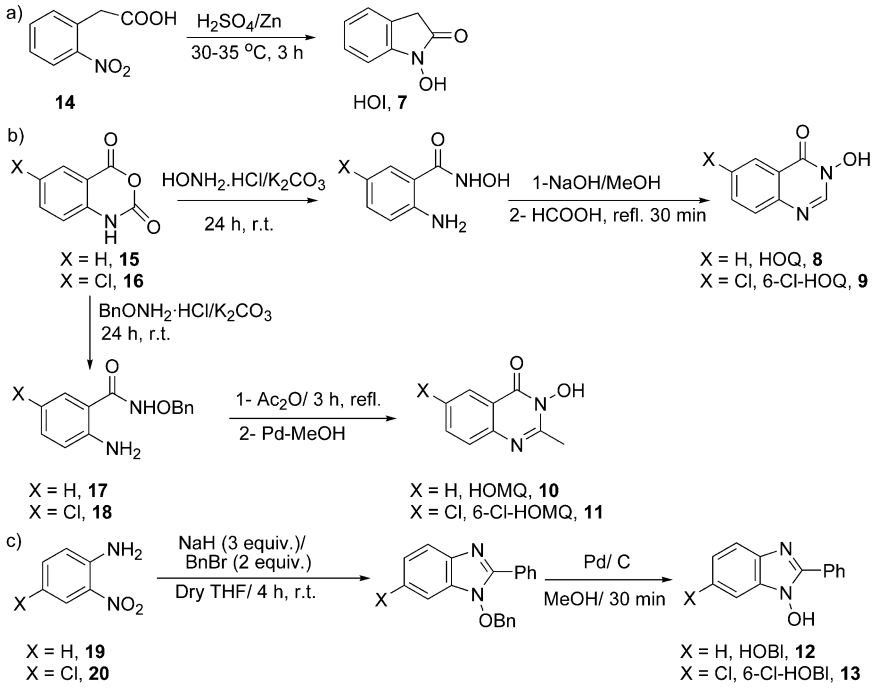
Compound **7** was prepared by reductive cyclization of (2-nitrophenyl)acetic acid (**14**) in the presence of Zn/H_2SO_4. Compounds **8**–**11** were prepared by reaction of substituted isatoic acid derivatives **15**–**18**, respectively, with hydroxylamine or benzyl derivatives, followed by reaction with $(HCO)_2O$ or $(AcO)_2O$. Compounds **12**[10] and **13** were prepared by reaction of *o*-nitroaniline derivatives **19** and **20** with NaH, followed by addition of benzyl bromide in dry THF, followed by catalytic hydrogenation with Pd/C in MeOH (Scheme 1). All these compounds were obtained with good to excellent yields (45–85%, yields not optimized) and characterized by NMR (^1H, ^{13}C) spectroscopy and elemental analysis (Table 1).

Table 1. Yield and racemization during the formation of Z-Phg-Pro-NH_2 in DMF at room temp. by using distinct additives (solution-phase synthesis). HPLC system, linear gradient over 30 min of 0.045% TFA in ACN and 0.036% aqueous TFA from 2:8 to 1:1; flow rate 1.0 min^{-1}, detection 220 nm, Waters Symmetry C_{18} columns (5 mm, 4.6 × 150 mm), Rt_{LL} = 26.01 min, Rt_{DL} = 27.40 min.

Entry	Coupling reagent	Yield (%)	DL (%)
1	HOI (7)/DIC	80.0	1.6
2	HOI (7)/DIC[a]	81.4	3.3
3	HOQ (8)/DIC	75.6	38.1
4	6-Cl-HOQ (9)/DIC	74.9	32.9
5	HOMQ (10)/DIC	76.3	36.1
6	6-Cl-HOMQ (11)/DIC	76.1	31.2
7	HOMQ (10)/DIC[a]	76.5	39.4
8	6-Cl-HOMQ (11)/DIC[a]	78.9	34.0
9	HOBI (12)/DIC	87.2	19.5
10	6-Cl-HOBI (13)/DIC	88.0	16.0
11	HOAt (4)/DIC	81.4	3.3
12	HOBt (1)/DIC	81.9	9.3

[a] 2 min preactivation.





Scheme 1. Synthesis of the *N*-hydroxy derivatives.

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To run a preliminary study of the performance of these new additives in carbodiimide-based coupling, we chose to synthesize the dipeptide Z-Phg-Pro-NH_2 model in solution by using DIC.

The phenylbenzimidazole derivatives (**12**,**13**) (Entries 9, 10) gave better coupling yields than HOBt and HOAt (Entries 11, 12). Furthermore, the coupling yield for *N*-hydroxyindolin-2-one (**7**) (Entries 1, 2) was similar to that of HOBt and HOAt (Entries 11, 12). In contrast, the remaining additives showed slightly worse performance than the parent compounds. Regarding racemization-suppressing properties, HOI (**7**) (Entries 1, 2) gave an excellent result, whereas HOBI (**12**) and 6-Cl-HOBI (**13**) (Entries 9, 10) showed poorer performance, but clearly better than the rest of the coupling additives tested. Again, as with the HOBt/Cl-HOBt system, the chloro derivatives gave better results than the parent compounds (Entries 4, 6, 8, 10 versus Entries 3, 5, 7, 9).

Conclusions

Although the model peptide is highly demanding in terms of racemization, HOI (**7**) and even 6-Cl-HOBI (**13**) are worth considering as potential substitutes for HOBt derivatives.

Experimental Section

General: TLC was performed on silica plates (8 × 4 cm) from Albet by using suitable solvent systems and visualization by a Spectroline UV Lamp Model CM-10 (254 nm). Melting points were obtained in open capillary tubes with a Gallenkamp Sanyo melting point apparatus and are uncorrected. NMR spectra were recorded with a Varian Mercury 400 MHz spectrometer at room temperature (r.t.). All solvents used for recrystallization, extraction, column chromatography and TLC were commercial grade, distilled before use and stored under dry conditions. Model peptide Z-Phg-Pro-NH_2 was analyzed according to previously described methods.[3] *N*-Hydroxyindolin-2-one (HOI, **7**),[11] 3-hydroxyquinazolin-4-one (HOQ, **8**),[12] 3-hydroxy-2-methylquinazolin-4-one (HOMQ, **10**),[13] and *N*-hydroxy-2-phenylbenzimidazole (HOBI, **12**)[14] were synthesized as described in the literature.

6-Chloro-3-hydroxyquinazolin-4-one (6-Cl-HOQ, 9): Synthesized according to a similar strategy described for the parent compound.[12] We obtained 0.71 g (36.6%) (from a 10 mmol reaction) of the product as a light brown solid. M.p. 268–269 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 7.74 (d, 1 H), 7.85 (dd, 1 H), 8.09 (d, 1 H), 8.56 (s, 1 H), 12.02 (s, 1 H, OH, exchangeable with D_2O) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 124.44, 125.44, 130.38, 132.06, 134.78, 146.27, 147.03, 157.01 ppm. $C_8H_5ClN_2O_2$ (196.5): calcd. C 48.88, H 2.56, N 14.25; found C 49.10, H 2.38, N 14.50.

6-Chloro-3-hydroxy-2-methylquinazolin-4-one (6-Cl-HOMQ, 11): Synthesized according to a similar strategy as that described for the parent compound.[13] 2-Amino-5-chloro-*N*-hydroxybenzamide (1.86 g, 10 mmol) was suspended in triethylamine (1.3 mL, 10 mmol), and then HOAc (10 mL) was added, followed by AcCl (1 mL) at room temp. The reaction mixture was stirred at room temp. for 1 h, and then H_2O (20 mL) was added. The precipitate was collected by filtration, washed with cold water, and dried to give the pure product [yield 1.69 g (80.3%)]. M.p.183–185 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 2.11 (s, 3 H, CH_3), 7.63 (dd, 1 H), 7.87 (d, 1 H), 8.45 (d, 1 H), 10.93 (s, 1 H, OH) ppm. ^{13}C NMR([D$_6$]-DMSO): δ = 25.57, 119.17, 122.56, 126.83, 130.80, 134.21, 140.18, 168.83, 169.26 ppm. $C_9H_7ClN_2O_2$ (210.6): calcd. C 51.32, H 3.35, N 13.30; found C 51.60, H 3.55, N 13.52.

6-Chloro-*N*-hydroxy-2-phenylbenzimidazole (6-Cl-HOBI, 13): Synthesized according to a similar strategy as that described for the parent compound.[14] M.p. 262–264 °C. ^{1}H NMR ([D$_6$]DMSO): δ = 7.25 (dd, 1 H), 7.53–7.57 (m, 4 H, Ph), 7.68 (d, 1 H), 8.20–8.25 (m, 2 H), 12.19 (s, 1 H, OH) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 123.72, 125.90, 130.35, 131.35, 132.36, 133.87, 134.88, 135.58, 136.35, 147.34, 148.17, 159.99, 165.26 ppm. $C_{13}H_9ClN_2O$ (244.68): calcd. C 63.81, H 3.71, N 11.45; found C 64.07, H 3.92, N 11.67.

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra of the compounds prepared.

Acknowledgments

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