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New Active Esters and Coupling Reagents Based on Pyrazolinones

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Abstract

Enol esters of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one [Mpp have surprisingly high reactivity[1]. Evaluation of 1- and 3-substituted derivatives showed that *1-(4'-nitro)-* variations were of comparable activity to the most reactive esters used for peptide synthesis[2]. Furthermore, they were crystalline, remarkably stable, selective to aminolysis, and possessed self indicating properties. Electron withdrawing substituents in th 3-position give even more reactive esters, which perform wel in solid-phase peptide synthesis studies. Selections for all Fmoc-L amino acids have been rigorously evaluated in controlled coupling experiments involving sterically and conform ationally difficult sequences. For Fmoc-L-Ser (tBu) and Fmoc Thr (tBu) esters, the optimal enol esters are formed from the novel 3-unsubstituted 1-(4'-nitrophenyl)-1H-pyrazol-5(4H)-on [Hpp]. In these cases, the bulk of the 3-substituent outweigh any advantage provided by additional electron withdrawing groups. A combination of this selection with 1,3-bis(4'-nitrophenyl)-1H-pyrazol-5 (4H)-one [Bnp] provides a practically useful set of esters for high efficiency peptide synthesis. Nev routes to these pyrazolinones are described. The esters are readily prepared from the corresponding uronium salts, whic also serve as useful coupling reagents in their own right.

Novel Highly Active Fmoc-Amino Acid-OXpp Esters

Previous work[1] showed that the substituted 5-hydroxy-1-phenylpyrazole esters, ONpp and OPnp (see Fig. 1, c = NO_2, R3 = CH_3 or Ph, respectively, R4=H) were not only highly active but were also nicely crystalline, stable on storage, and possessed self-indicating properties. This communication describes studies on a wide range of variations, referred to as Xpp esters, which are listed in Table 1. To better assess the relative activities of these highly active compounds the original competition method has been modified so that the Fmoc-Val test ester and Fmoc-Ile-ONpp ester (rather than OPFP) are mixed in equimolar ratios and coupled t a H-Gln(Tmob)-Ala polystyrene support. The more valine incorporated relative to isoleucine, the more activ the test ester.

The results show clearly the very high activity of these compounds. Note that the Dpp ester has a comparab activity to the OPFP ester; the most active "useful" Xpp, Bnp, being ≈ 20 times more active, and other candidates, which appear too acidic in nature to allow complete coupling in the absence of base, such as Tnp and NCNp, are even more active. As expected the results show that electron withdrawing substitutions at the 3 position of the pyrazole ring lead to increasing activity, although this is not always directly related. For example, NClp is less active than the unsubstituted Pnp. Clearly electron withdrawing substituents are better favored at c in R1, Pnp being far more active than the structural isomer Ppn, and Clnp less active than NClp.

A variety of substitutions are possible at position R4. However, comparison of the activity of EtHp and Necp show substitutions here to be less effective than at R3, and this may be explained by the increase in steric hindrance associated with a substitution closer to the site of nucleophilic attack. It is also possible that anchiomeric assistance by hydrogen bonding interactions plays a significant role in the remarkably high activity of these esters, and steric influences would also be important here.

Of considerable interest is the high activity of the thioester of 2-mercaptobenzothiazole (SBt ester)[6], being markedly more active than the 2,4-dinitrophenyl ester. This result reflects the well known greater susceptibility of thioesters than esters to nucleophiles. Although not self-indicating these appear to offer a viable, non-toxic and economical alternative to current selections.

At an intermediate stage in these studies we believed that OBnp and OPnp esters, when used in combination would provide a practically useful set of derivatives. We prepared many of these esters and found that they coupled excellently in the synthesis of several test sequences. The data provided in Table 1, and the time course studies of coupling to the IATGKVLTY sequence, indicate that highly active esters do not necessarily couple to completion in an acceptable time. Besides acidity, steric hindrance also plays a role. These findings lead to the hypothesis that a hydroxypyrazole ester lacking substitution both at positions 3 and 4 would be a superior selection. Consequently, Hpp was prepared (Fig. 1), and, in agreement with these suppositions its esters were found to have significantly higher activity in both competition and peptide coupling assays.

Hpp esters are, therefore, finely tuned to minimize steric effects, and to balance the acidity of the leaving group to achieve high activity, yet will not protonate resin-bound amino groups so completely as to slow late stages of coupling. In the presence of base, the more highly active derivatives, such as Necp, provide high efficiency and rapid coupling. These esters may find application under circumstances where a potential for racemization is absent.

Table 1: Properties and Relative Reactivities of Various Fmoc-Valine OXPP Esters

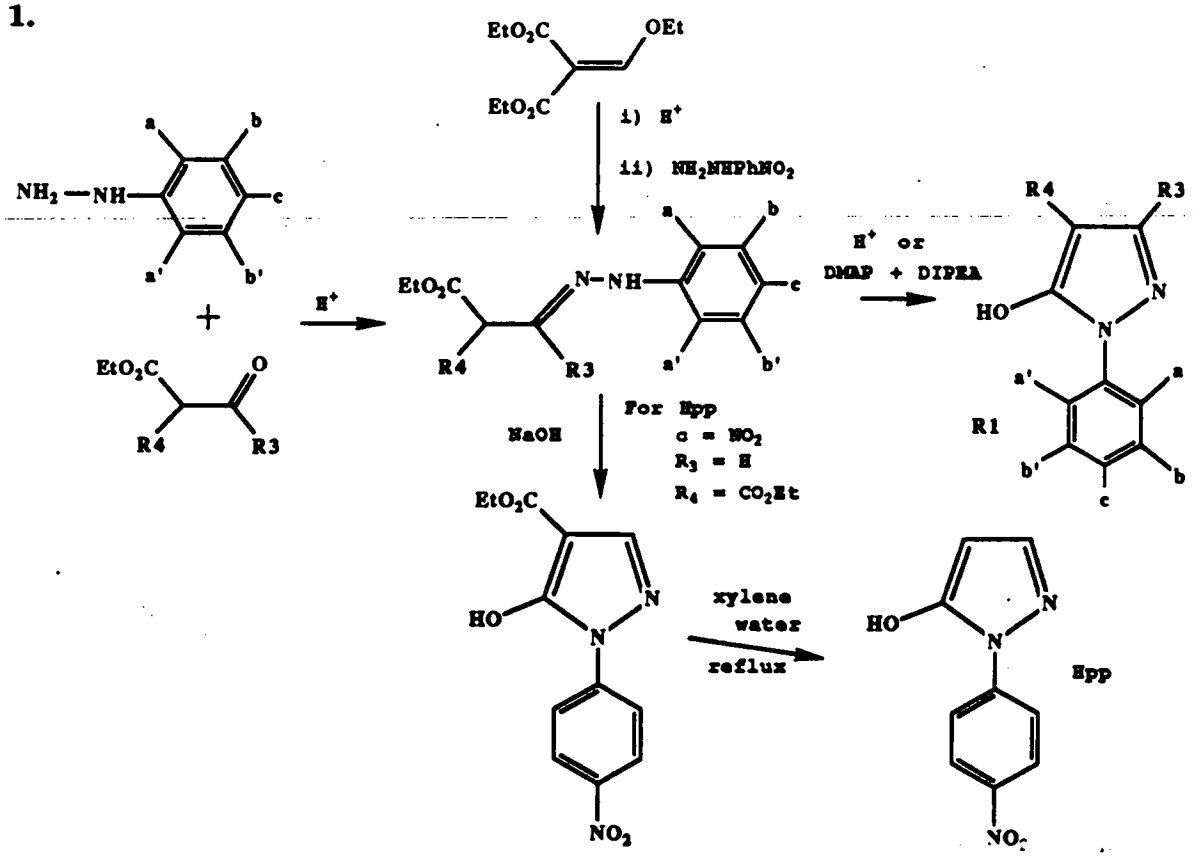
ESTER	R1 a	R1 b	R1 c	R3	R4	SELF-INDIC.	m.p.	Comp. V/I	Assay Rel. Act.
Npp	H	H	NO_2	CH_3	H	+	195-197	1.36	1.00
Hpp	H	H	NO_2	H	H	+	167-169	1.67	1.23
Mpp	H	H	H	CH_3	H	–	151-152	0.10	0.07
Dpp	H	H	H	Ph	H	–	147-149	0.29	0.21
Ppn	H	H	H	$PhNO_2$	H	+	176-177	0.71	0.52
Pnp	H	H	NO_2	Ph	H	+	168-171	3.16	2.32
NClp	H	H	NO_2	Ph-Cl	H	+	186-189	3.04	2.24
Bnp	H	H	NO_2	$PhNO_2$	H	+	168-170	4.50	3.31
Tnp	NO_2	H	NO_2	$PhNO_2$	H	+	103-105	8.16	**
Dnp	NO_2	H	NO_2	Ph	H	+	79-95	3.74	**
Clp	H	H	Cl	Ph	H	–	112-113	0.58	0.42
ClNp	H	H	Cl	$PhNO_2$	H	+	164-167	1.42	1.04
DClp	Cl	H	Cl	Ph	H	–	146-149	0.20	0.15
EtHp	H	H	NO_2	H	CO_2Et	+	131-134	2.09	***
Necp	H	H	NO_2	CO_2Et	H	+	161-165	9.10	***
Nacp	H	H	NO_2	$CONH_2$	H	+	foam	4.34	3.19
NCNp	H	H	NO_2	CN	H	+	foam	13.85	****
DNP	2,4-dinitrophenyl ester					weak	112-113	2.07	1.52
PFP	pentafluorophenyl ester					–	122-127	0.24	0.18
SBt	2-mercaptobenzothiazole thioester					–	129-131	3.98	2.98

Asterisks indicate coupling incomplete in assay: ** ≈90%; *** ≈80%; **** ≈50%; R1, R3, R4, a, b, c as in Figure 1.

Pyrazolinone Synthesis

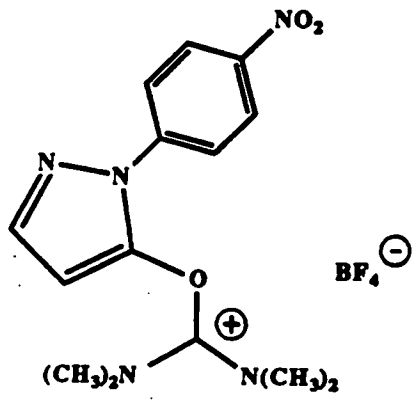
A variety of novel 1-phenylsubstituted pyrazolinone derivatives have been prepared. The standard method c Knorr[3] (Fig. 1) can be used for the majority of the syntheses. For di- or tri- nitro substituted compounds, only the intermediate hydrazone is obtained even under forcing acidic conditions (cf. ref. 4). Conversely, in the presence of 1 equivalent of diisopropylethylamine and acetonitrile, high yields of conversion result in these cases. Formation of the 3-unsubstituted derivative Hpp can not be achieved by these methods, but can be obtained in high yield by the simple route outlined (Fig. 1).

Figure 1.



Coupling Reagents

Figure 2.



HppTU

Variants of the tetramethyluronium coupling agent HBTU[5] have been prepared incorporating 1-phenylpyrazolinone derivatives instead of 1-hydroxybenzotriazole. These function as effective coupling reagents in their own right, and have been used for the syntheses of several peptides. HOBt is superior to the 1-phenylpyrazolinones as an additive for these couplings, as well as for carbodiimide and active ester reactions. The variant of the BOP reagent has yet to be obtained in a non-nucleophilic salt form. The tetra methyluronium salts are exceedingly useful in the synthesis of the title active esters themselves. This route eliminates the need to use DCCI, and avoids problems associated with complete removal of dicyclohexylurea.

A Complete Selection of Novel Esters For Solid Phase Peptide Synthesis

Active esters of Fmoc-Ser(tBu) and Fmoc-Thr(tBu) are notoriously difficult to crystallise, and tend to be of poor stability. We, therefore, prepared several Xpp esters of these, and preliminary testing indicated that OPnp and OBnp derivatives, respectively, were an outstanding selection. These were then tested in a highly difficult coupling, to H-Ile-Ala-Thr(tBu)-Gly-Lys(tBoc)-Val-Leu-Thr(tBu)-Tyr(tBu)-polystyrene resin, and found to give somewhat poorer incorporations than the standard ODhbt selections. These comparisons showed a trend different from that shown in the competition assay: Npp > Bnp = Pnp > Tnp. Since the bulk of R3 wa implicated, the novel pyrazolinone Hpp was prepared, and active esters tested in both the competition assay and the difficult coupling. The Hpp esters were found to be superior to Npp esters in both trials. The Hpp esters of Ser and Thr are crystalline, stable, have self-indicating properties, and couple more efficiently than d the far less stable and non-crystalline ODhbt esters. These findings are substantiated by comparison synthese of peptide T (Figure 3 shows the HPLC profiles of products obtained under various conditions). When coupling is performed at low concentrations for brief intervals the Hpp esters gave superior coupling efficiencies and a better quality product. The application of Hpp esters to a complete range of amino acids has been evaluated. In most cases significantly better coupling efficiencies are obtained than with the standard OPFP and ODhbt esters (Table 2). Note that the Bnp derivative of Fmoc-Ile is more soluble and as efficient as Fmoc-Ile-OHpp, and is, therefore, preferred. For histidine the SBt thioester provides a viable alternative to th OPFP ester. Because of its marginally superior activity the OPFP ester may still be preferred. Interestingly, and for reasons which elude us, our attempts to prepare Xpp esters of Fmoc-His(tBoc), Fmoc-His(Trt) and Fmoc-His(Bum) have been unsuccessful.

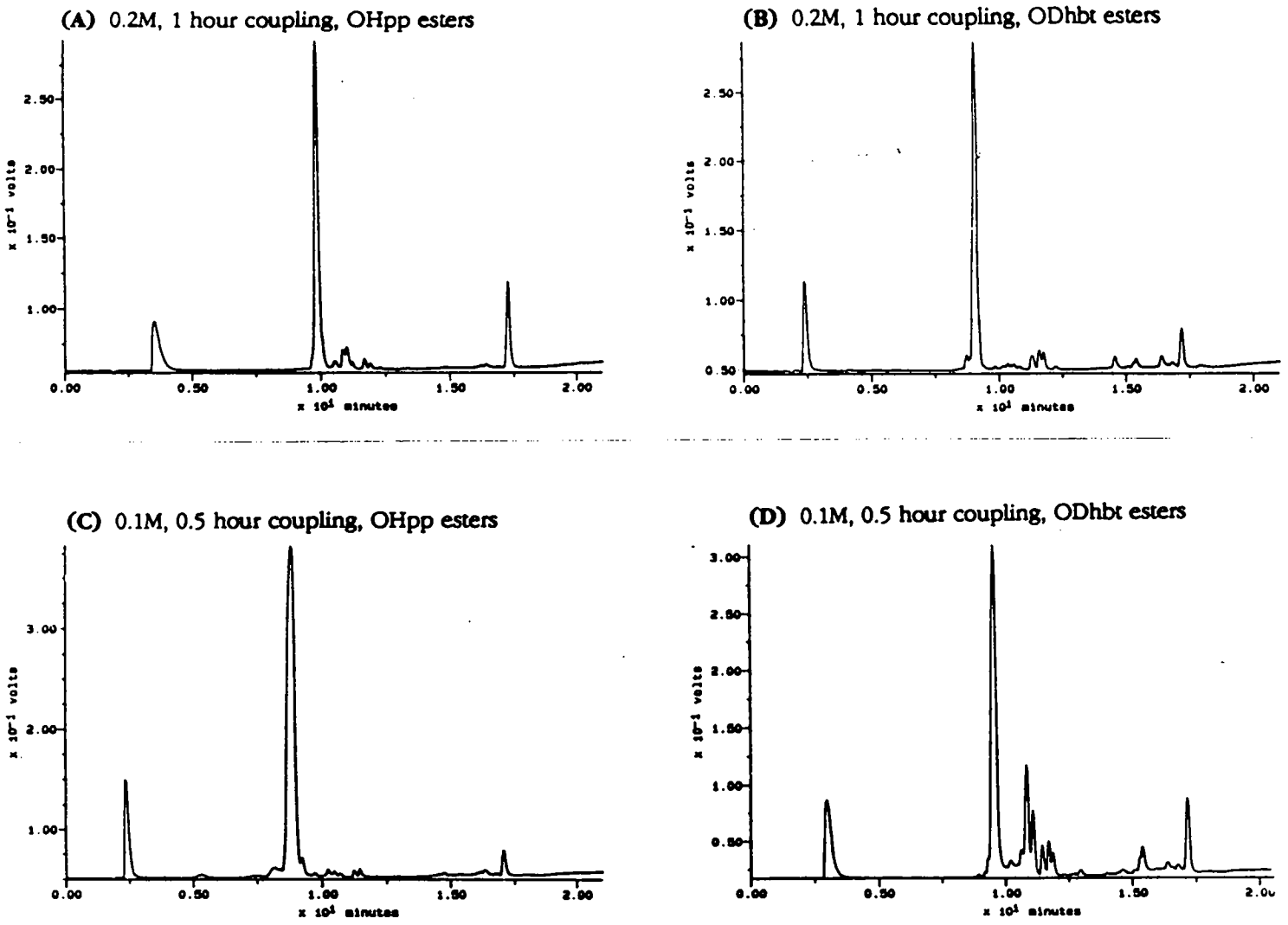
Table 2: New Active Esters of Fmoc-Amino Acids

AA	ESTER*	M.P. (°C)	RELATIVE EFFICIENCY**	AA	ESTER*	M.P. (°C)	RELATIVE EFFICIENCY**
Ala	Hpp	174-177	1.00	Leu	Hpp	156-159	>1.0
Arg(Pmc)	Hpp	foam	≈ 1.00	Lys(tBoc)	Hpp	158d	1.04
Asn(Trt)	Hpp	149-150	≈ 1.0	Met	Hpp	153-155	>1.0
Asp(OtBu)	Hpp	86-95	1.07	Phe	Hpp	164-167	>1.0
Cys(StBu)	Hpp	87-92	n/d	Proline	Hpp	135-138	1.08
Gln((Trt)	Hpp	65d	≈ 1.0	Ser(tBu)	Hpp	103-106	1.33
Glu(OtBu)	Hpp	132-135	1.10	Thr(tBu)	Hpp	99-103	1.11
Gly	Hpp	156-158	1.29	Trp	Hpp	151-153	>1.05
His(tBoc)	SBt	broad	0.88	Tyr(tBu)	Hpp	135-138	1.00
Ile	Bnp	190-192	1.28	Val	Hpp	167-169	1.09

* Refer to Table 1 for definitions.

** Relative Coupling Efficiency was determined by coupling to IATGKVLTY at 0.2M for 1 hour (Ser, Thr 0.1M for 0.5 hour) and comparing incorporation to that obtained with corresponding OPFP+HOBT or ODhbt ester [note: Asn(Trt) and Gln(Trt) compared to side-chain un-protected OPFP's, Arg(Pmc) compared to Arg(Mtr)-OPFP], TATGKTETY used for Ile comparisons.

Figure 3. Comparative syntheses of ASTTTNYT- amide with Ser and Thr active esters



Conclusions

These results show that Fmoc-amino acid active esters derived from the novel 5-hydroxy-1-(4'-nitrophenyl)-pyrazole, Hpp, are of high intrinsic reactivity. They give superior results to current selections in the solid-phase synthesis of complex sequences involving sterically hindered couplings. Hpp esters are self-indicating, and their properties (e.g. high crystallinity, stability and non-toxic nature) render them highly suitable for commercialization. A complete selection of novel esters for all 20 naturally occurring amino acids has been made.

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