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RESIN-SUPPORTED LABELING REAGENTS

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Abstract: Resin-supported fluorescein, coumarin, acridinium, and biotin active esters were prepared from a new *N*-hydroxysuccinimidyl resin in high yield. The active esters were used to prepare representative conjugates with estriol, thyroxine, phenytoin, and desipramine haptens without need for purification beyond removal of the spent resin.

Assays for substances of clinical significance often employ a conjugate bearing an easily detectable tag.[1,2] Traditional solution-phase preparation of such conjugates can be problematic. A reactive form of the label must first be prepared and purified (an active ester, for instance). In an earlier report we have described the preparation and use of resin-bound carbodiimides for the preparation of active esters to simplify this step.[3] In that case, the urea by-product and unreacted acid were sequestered on the resin leaving pure active ester in solution. Even so, subsequent conjugation with the analyte was seldom quantitative; the final product usually required careful purification to remove any unattached tag or other reaction by-products that would potentially interfere with the assay. We have since prepared *N*-hydroxysuccinimidyl (NHS) active ester resins to address these issues in a general synthesis of amides.[4] To our knowledge no analogous resin-supported labeling reagents have been used in the preparation of conjugates used in clinical assays. Conjugates labeled using soluble NHS active esters of fluorescein,[5,6] coumarin,[6] *N*-sulfonyl-acridinium-9-carboxamide,[3,7–10] and biotin[11] are well known. For this communication the corresponding resin-supported active esters were prepared and subsequently conjugated with representative haptens used in clinically important immunoassays.

As illustrated in the Scheme, the solid-phase *N*-hydroxysuccinimidyl 6-carboxyfluorescein active ester (**3**) was prepared by reacting the NHS resin (**1**, 1 mmol/g) with a threefold excess of 6-carboxyfluorescein (**2**) and ethyl (3-dimethylaminopropyl) carbodiimide (EDC) in dimethylformamide (DMF). The resulting resin was collected by filtration, washed extensively with DMF, then dried under high vacuum to give **6** as a free flowing powder. Similar transformations were performed with coumarin acid **3**, *N*-sulfonyl-acridinium-9-carboxamide carboxylic acid **4**[12] and biotin **5** to afford the corresponding resin-supported active esters **7–9**.

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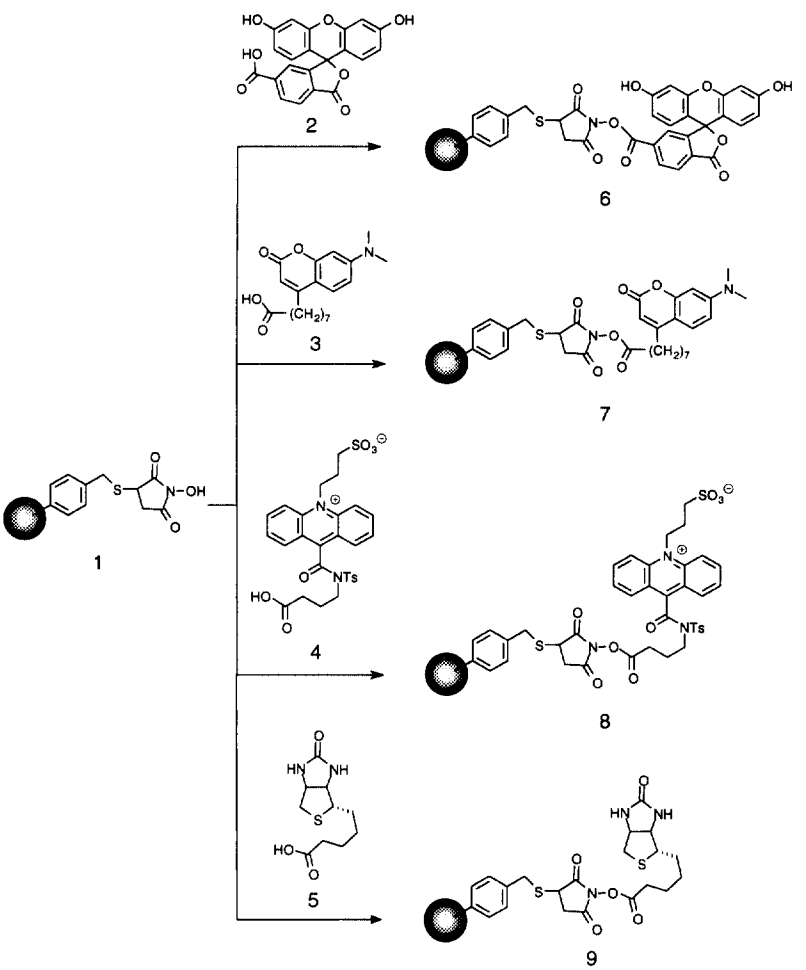
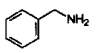
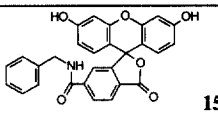
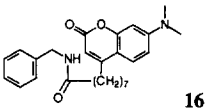
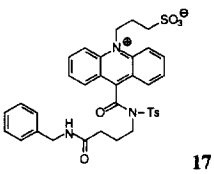
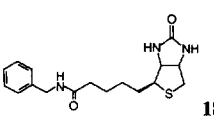
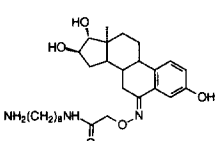
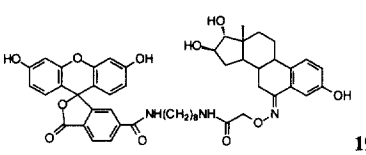
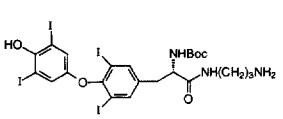
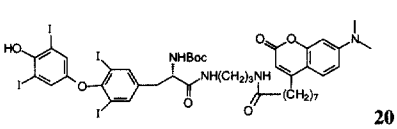
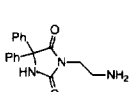
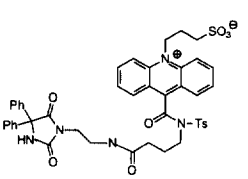
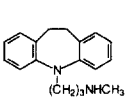
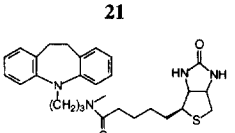
The reaction of the resin-supported labeling reagents with representative amines is summarized in the Table. Each amine was added to a sixfold excess of active ester resin suspended in DMF then stirred for 20 h. The spent resin was then collected by filtration and washed with DMF. Evaporation of the combined filtrates afforded the corresponding amides. When each resin was tested using benzylamine (**10**) the yields of benzylamide ranged from 87 to 98%. The amine-substituted estriol[13] derivative (**11**) was acylated by 6-carboxyfluorescein NHS resin **6** to produce the fluorescent estriol probe **19** in 92% yield. The coumarin NHS resin (**7**) afforded the fluorescent thyroxine conjugate (**20**)[14] in similar yield (95%) under the same conditions. Acridinium salts such as **4** are known to add nucleophiles, including amines, to the 9-position of the acridinium ring.[15,16] This competing reaction may explain the low yields that are often obtained when conjugating the

Scheme. *Synthesis of resin-supported labeling reagents*[a]



[a]*Reaction conditions*: EDC, DMF, ambient temperature, nitrogen atmosphere, 16–18 h.

Table. *Conjugation using resin-supported labeling reagents*

Resin-supported labeling reagent	Amine (**10–14**)	Labeled Conjugate (**15–22**)[18]	% Yield[a]
6	 **10**	 **15**	87
7	**10**	 **16**	98
8	**10**	 **17**	91
9	**10**	 **18**	88
6	 **11**[13]	 **19**	92
7	 **12**[14]	 **20**	95
8	 **13**[17]	 **21**	88
9	 **14**	 **22**	98

[a]Isolated yields after filtration and evaporation.

acridinium acid with amines using a 1:1 stoichiometry in solution. It was interesting to note then, that when the resin-bound acridinium active ester (**8**) was used *in excess* of the phenytoin hapten,[17] the chemiluminescent

phenytoin tracer (**21**) was still isolated in 88% yield. More sterically hindered secondary amines reacted well with the resin-supported active esters. This was exemplified by the reaction of the tricyclic antidepressant drug, desipramine (**14**), with the active ester resin (**9**) that afforded a 98% yield of the biotinylated conjugate (**22**).

In summary, the resin-supported labeling reagents **6–9** were straightforward to prepare from the NHS resin **1** and the corresponding carboxylic acid. Further reaction with simple amines or more complex haptens efficiently led to the amides **15–22**. Analytical reversed-phase HPLC analysis indicated that the purity of the labeled conjugates ranged from 91–99%[19] without any purification beyond filtration of the resinous reagent and evaporation of the solvent. Work is currently underway to extend the use of resin-supported labeling reagents to the preparation of other conjugates with analytes including drugs, peptides, proteins and nucleic acids.

References and Notes

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12. Adamczyk, M.; Chen, Y.-Y.; Mattingly, P. G.; Pan, Y. *J. Org. Chem.* **1998**, *63*, 5636.
13. Hapten **11** was prepared from estradiol-6-CMO (Steraloids, Wilton, NH): (a) EDC/NHS; (b) 1,8-diaminooctane.
14. Flentge, C. A.; Kirkemo, C. L. *Eur. Patent Appl.* EP 108403 A2 840516.
15. Adamczyk, M.; Fishpaugh, J. R.; Gebler, J. C.; Mattingly, P. G.; Shreder, K. *Eur. Mass Spec.* **1998**, *4*, 121.
16. Biwersi, J.; Tulk, B.; Verkman, A. S. *Anal. Biochem.* **1994**, *219*, 139.
17. Hapten **13** was prepared from 5,5-diphenylhydantoin (Aldrich): (a) KOH/EtOH; (b) 1,2-dibromoethane; (c) ammonium hydroxide.
18. All compounds gave satisfactory ^{1}H NMR and mass spectral analyses. Purity was determined by analytical HPLC [Waters μBondapak C_{18} column, eluting with acetonitrile/0.05% aq trifluoroacetic acid with UV detection at 254 nm.]
19. The major impurity was residual solvent. Minor impurities sometimes carried over from the starting amine. No active ester resin decomposition products were seen.