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A NEW LINKER FOR PRIMARY AMINES APPLICABLE TO COMBINATORIAL APPROACHES.

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Abstract: We describe a new linker molecule (ADCC-linker) for the attachment of primary amines to solid support. The linker is stable towards acidic conditions (eg.TFA) basic conditions (eg.piperidine, DBU) as well as towards uronium type coupling reagents. The amine can be quantitatively released from the support using a 2 % hydrazine in DMF solution.

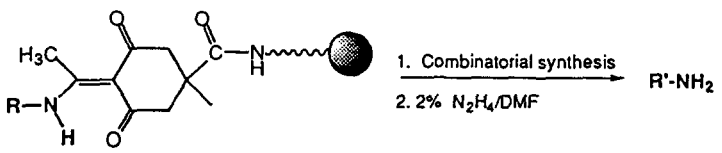
In drug discovery combinatorial approaches are attractive because they speed up the process of lead finding and the optimization of a lead compound up to the final drug as compared to traditional medicinal chemistry.[1,2] The preferred method for combinatorial chemistry is synthesis on a solid support.

A critical element in solid phase synthesis is the availability of suitable linker units between the target molecule and the polymer. The linker molecule should be stable to the conditions of the combinatorial synthesis but it should be possible to cleave the compound from the support without affecting it. Furthermore, no contamination of the desired product should occur during the cleavage step. Ideally, cleavage reagents should be sufficiently volatile so they may easily be removed from the released product by evaporation.

For the attachment of carboxyl functions a whole range of linker molecules has been developed, almost exclusively originating from the field of peptide chemistry. Most of them are cleavable under acidic conditions. Due to the emerging combinatorial approaches alternative linkers have been developed. They allow the cleavage of the combinatorial compound from the support under oxidative,[3] photochemical [4] or basic conditions following a methylation reaction [5,6] as well as release by fluoride ion.[7] Regardless of the procedure all the compounds cleaved from the support will contain a carboxyl- or a carboxamide group.

To further the diversity of combinatorial chemistry there is a need for linkers that permit the attachment and the release of other functional groups to the support. To this end Ellman has described a linker for the hydroxyl group based on the tetrahydropyranyl protecting group.[8] A linker for primary amines was recently reported in the synthesis of head-to-tail cyclized peptides and based on a novel active carbonate resin. [9]

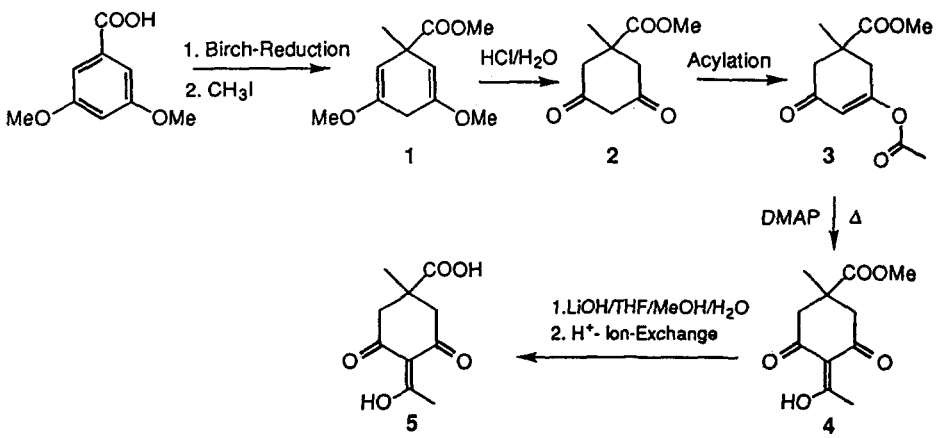
Here we would like to report on a new linker for primary amines which is compatible with combinatorial synthetic strategies (*Scheme1*). The linker molecule is based on a protecting group derived from acetyldimedone as developed by Bycroft [10] and applied to the protection of the ε- amino function of lysine in peptide synthesis.



Scheme 1

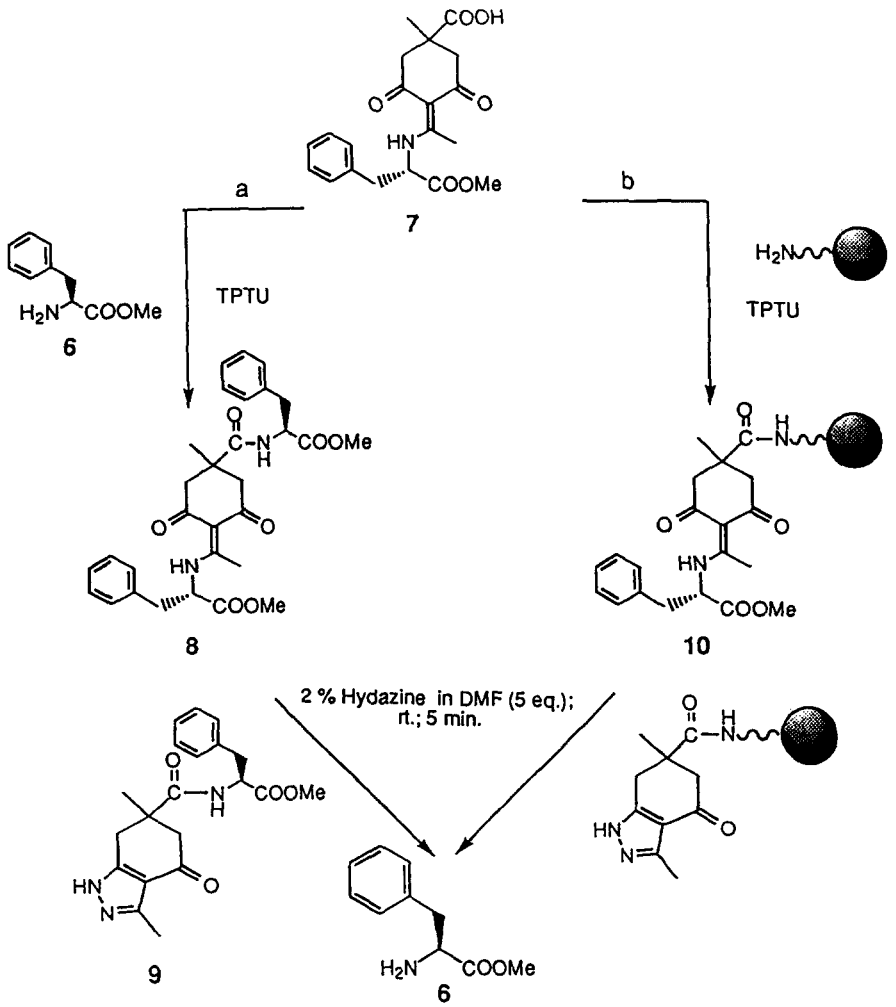
On the one hand it is stable towards acidic conditions and on the other hand it is also stable towards basic conditions (Piperidine; DBU). Yet, it can be cleaved off quantitatively by the application of 2% hydrazine in DMF.[11] A further advantage of the linker is its complete stability towards uronium type coupling reagents used routinely in amide bond formations between carboxylic acids and amines.[12]

The synthesis of the linker molecule is described in *Scheme 2*. Birch reduction of 3,5-dimethoxybenzoic acid is followed by methylation to yield the methyl ester of the bis-enol derivative **1** (58%, two steps). Treatment of **1** with aqueous HCl results in the formation of the 3,5-dioxo-1-methyl-cyclohexane carboxylic acid methyl ester **2** (94%).



Scheme 2

Acylation of **2** yielded the O-acylated derivative **3** which was transformed on heating in the presence of DMAP to the desired 4-acetyl-3,5-dioxo-1-methyl-cyclohexane carboxylic acid methyl ester **4** (93%, two steps). Finally, saponification of the methyl ester and treatment with Ion-Exchanger (H^+) resulted in the quantitative formation of the desired linker molecule 4-acetyl-3,5-dioxo-1-methyl cyclohexane carboxylic acid (ADCC-linker) **5** as a crystalline compound.[13]



Scheme 3

In a control experiment (*Scheme 3, a*) compound **7**, which was obtained quantitatively by stirring the linker molecule **5** and phenylalanine methyl ester **6** in DMF, was coupled in solution for one hour to another molecule of L-phenylalanine methyl ester employing 1,1,3,3-tetramethyl-2-(2 oxo-1(2H)-pyridyl uronium tetrafluoroborate (TPTU) as coupling reagent to give after chromatography derivative **8** in a yield of 77%. Compound **8** was found to be stable towards 50% TFA in CH_2Cl_2 and towards 20% piperidine in DMF as indicated by TLC. Treatment of **8** with 2% hydrazine in DMF caused after 5 min. quantitative release of phenylalanine as well as the formation of the oxotetrahydro-1-H indazole derivative **9**.

Compound **7** was also attached with TPTU to amino-modified polystyrene beads (*Scheme 3, b*) using a 2.5 fold excess of **7**. Complete reaction was established by performing a Ninhydrin

test on a resin sample. Furthermore, FT-IR of the support material after the reaction clearly indicated formation of the amide bond in **10**.[14,15]

For the cleavage of the L-phenylalanine methyl ester from the support the material was shaken in a 2% hydrazine hydrate solution in DMF for 5 min under Argon. The support was filtered and washed several times with DMF. Evaporation of the combined DMF solutions resulted in the quantitative recovery of the phenylalanine methyl ester **6**. Signal bands corresponding to an ester function could not be detected in FT-IR of the support after cleavage, indicating complete release of the phenylalanine methyl ester.

In summary, we have developed a new linker for the attachment of primary amines to solid support materials. The linker is resistant to a range of basic and acidic conditions and yet, the amines can be released quantitatively with 2% hydrazine in DMF within 5 min. Application of the linker molecule in solid phase synthesis will be reported in due course.

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15. Abbreviations: DBU: 1.8-Diazabicyclo[5.4.0]undec-7-ene; DMAP: Dimethylaminopyridine; FT-IR: Fourier-Transform-Infrared-Spectroscopy; TFA: Trifluoroacetic acid; TLC: Thin layer chromatography.

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