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# A Universal Allyl Linker for Solid-Phase Synthesis.

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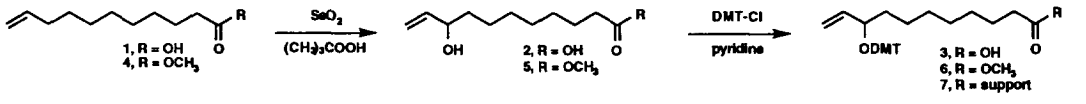
***Abstract***: We report synthesis of a universal allyl linker for solid-phase synthesis, 9-*O*-(4,4'-Dimethoxytrityl)-10-undecenoic (3), that has a reactive terminal double bond. Since allyl cleavage occurs under conditions orthogonal to those used during the solid-phase synthesis and deprotection of DNA or RNA fragments, this linker extends the range of post-synthetic manipulations that can be carried out without cleavage from the support, and means that this linkage could be used to construct affinity columns. Alternatively, it should be possible also to cleave fully protected molecules from the support if so desired. Copyright © 1996 Elsevier Science Ltd

Solid-phase synthesis of DNA and RNA fragments most commonly employs a succinate linkage between the 3'-terminal nucleoside and an amino functionalized support, although other linkages have been proposed for specific, generally limited, uses.[1,2] A particular deficiency of the succinate and other non-universal linkages is the need for preparation of specially derivatized 3' monomers, and an additional set of reactions, to load the 3' monomer onto the support. Moreover, the lability of the succinate linkage limits the types of post-synthetic manipulations that can be carried out on the support-bound nucleic acid fragment. RNA synthesis, for example, requires deprotection of the 2'-*O*-*tert*-butyldimethylsilyl (BDMS) group after deprotection of the amino groups. With a succinate linkage amino deprotection cleaves the RNA from the support. Once the RNA has been cleaved from the support it becomes more difficult to achieve the anhydrous conditions necessary for reaction with *tetra*-*n*-butylammonium fluoride (TBAF), and removal of the excess TBAF is time-consuming.[3] Triethylamine tris(hydrogen fluoride) is an alternative to TBAF[4] that is less sensitive to water,[5] and is more easily removed than is TBAF,[3] but may not be compatible with trityl-on purification of the RNA fragment.

We set out to design a linker that would be universal, and would be cleaved under conditions orthogonal to the other reactions used in nucleic acid synthesis. For this purpose, the Pd(0) mediated reactions of allyl groups appeared to be good candidates. Hayakawa and Noyori introduced the allyl group for phosphate protection in 1985,[6] and extended this methodology to the allyloxycarbonyl group for hydroxyl and amino protection.[7,8] There followed application to solid phase peptide synthesis where several different allylic linkers containing internal double bonds were reported.[9-11] We now report synthesis of an allylic linker with a more reactive terminal double bond.



The preparation the linker molecule, 9-*O*-(4,4'-dimethoxytrityl)-10-undecenoic (**3**), shown in Scheme 1, is a straightforward two step synthesis. The key step is a selenium dioxide oxidation[12] of a terminal alkenoic acid, in this case 10-undecenoic acid (**1**), or its methyl ester **4**, to give the corresponding allylic alkoxy derivatives **2** or **5** in about 75 % yield. The ester (**5**) is purified by distillation, while crude **2** is used directly in the next step. Tritylation of **2** or **5** to give the DMT derivatives **3** or **6** proceeds under standard conditions using 4,4'-dimethoxytrityl chloride in pyridine. While in principle many terminal alkenoic acids could be used in this synthesis, the low cost of **1**[13] make it particularly attractive. The allyl linker molecule **3**, after purification by silica gel chromatography, then can be attached to any amino or hydroxy functionalized support by a variety of standard procedures.[1] We use amino functionalized polystyrene/polyethylene glycol[14] in methylene chloride with DCC,[15] which gives loadings of about 170 μmole/g, as determined by trityl assay.

Automated DNA or RNA synthesis with support-bound **3** can be carried out by any of the standard nucleic acid synthetic methods. Importantly, for RNA synthesis desilylation with TBAF or TEA•3HF can be carried out while the RNA fragment is attached to the support, and the excess reagent removed simply by washing the support-bound RNA with appropriate solvents. In addition, the ammonia treatment will cleave DNA fragments at any depurinated sites so that the 5'-DMT portion of these fragments is removed, thereby significantly simplifying the purification.[16] Cleavage of the nucleic acid fragment from the support can be effected under the conditions reported by Noyori[8] to give the oligomer as its 3'-phosphate.[17]

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