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SYNTHESIS OF CHEMOREVERSIBLE PRODRUGS OF *ARA*-C

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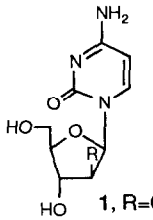
Abstract: The synthesis of N^4-peptidyl- derivatives of *ara*-C (**1**) is described. In these derivatives, the active drug is released by an intramolecular cyclization process with formation of a six-membered heterocycle. No enzymatic or solvolytic conversion is necessary. NMR studies of the rates of cyclization in basic and acidic environment are discussed.

In the field of cancer chemotherapy, nucleoside analogs have traditionally been of high significance.[1] 1-(β-D-arabinofuranosyl)cytosine (*ara*-C, **1**, R=OH) is one of the most effective drugs for the treatment of human acute myeloblastic leukemia.[2-4] *In vitro*, *ara*-C inhibits DNA polymerase α with K_i values in the micromolar range.[5] In mammalian cells, however, the IC_{50} for DNA synthesis inhibition is considerably decreased,[6,7] and most of the *ara*-C that is incorporated into DNA is found in internucleotide linkages rather than at the 3'-termini. Therefore, several mechanisms ultimately seem to contribute to the observed extraordinary potency of *ara*-C *in vivo*.[8-10]

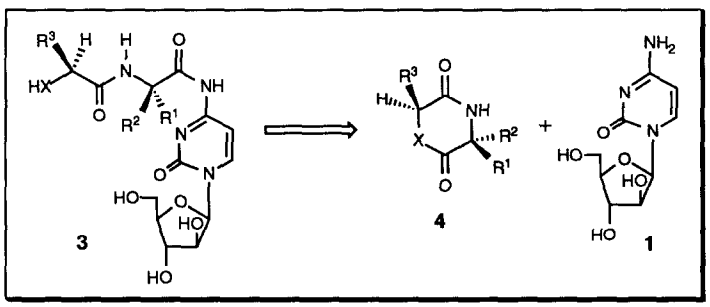


1, R=OH
2, R=N_3

Limitations in the application of *ara*-C are: a short half-life in plasma due, in part, to deamination to inactive *ara*-U by cytidine deaminase, development of resistance, ineffectiveness on solid tumors, and severe toxic side effects caused by large doses of the drug.[11-13] As with anti-neoplastic agents in general, curative doses cannot be administered without unacceptable side effects.[14] To overcome these problems, derivatives[15] and prodrugs[16-22] of *ara*-C have been synthesized.

2'-Azido- and 2'-amino-2'-deoxy-*ara*-C (Cytarazid; **2**, R=N_3 and Cytaramin) are readily available[23] derivatives of *ara*-C resistant to deamination.[24] N^4-Palmitoyl-*ara*-C and several phospholipid conjugates of *ara*-CMP have also found to be more effective than *ara*-C.[25,26] We describe herein the synthesis of N^4-peptidyl- derivatives of *ara*-C (**3**, X = NH, O) that release the active drug by an intramolecular cyclization process with formation of a six-membered heterocycle (Scheme 1).

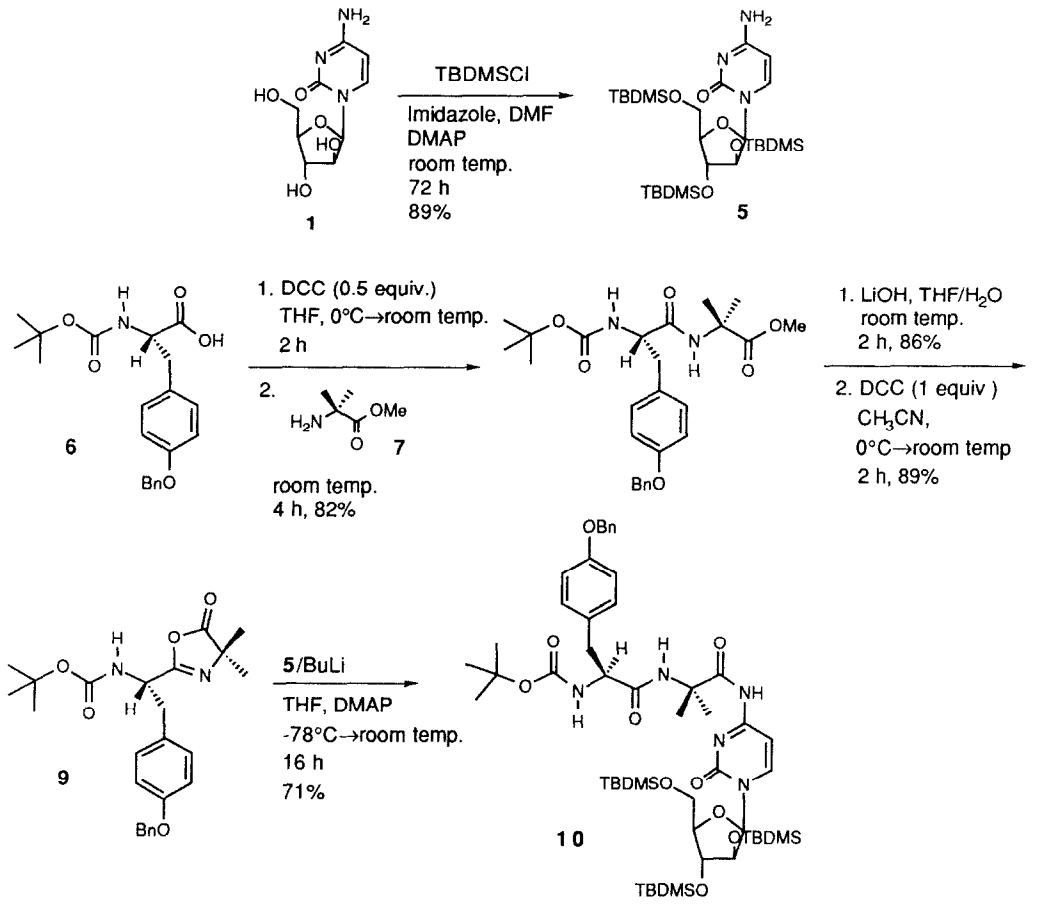
Scheme 1.



The N^4-substitution circumvents degradation by cytidine deaminase,[25] and the use of nonproteinogenic α,α-disubstituted amino acids such as 2-methylalanine (Aib) inhibits early deacylation by hydrolytic enzymes. The rate of cleavage of the amide bond in peptides with disubstituted amino acids is significantly reduced.[27] As a consequence of the Thorpe-Ingold effect,[28] however, the presence of an α,α-disubstituted amino acid also facilitates the cyclization and thus the intramolecular drug activation process considerably.[29]

Peptide bond formation at the N^4-position of *ara*-C is complicated by the intrinsically low nucleophilicity of the amino function and the presence of three arabinose hydroxyl groups that contribute to the low solubility of the compound in common organic solvents.[30-32] Initial attempts for the direct preparation of benzyloxycarbonylglycyl-*ara*-C from benzyloxycarbonyglycine and *ara*-C using the BOP coupling protocol[33] were indeed unsuccessful. Therefore, trisilylated nucleotide **5** was prepared by treatment of *ara*-C with 6 equiv of TBDMSCl and imidazole in DMF in the presence of 25 mol % of DMAP[34] (Scheme 2).

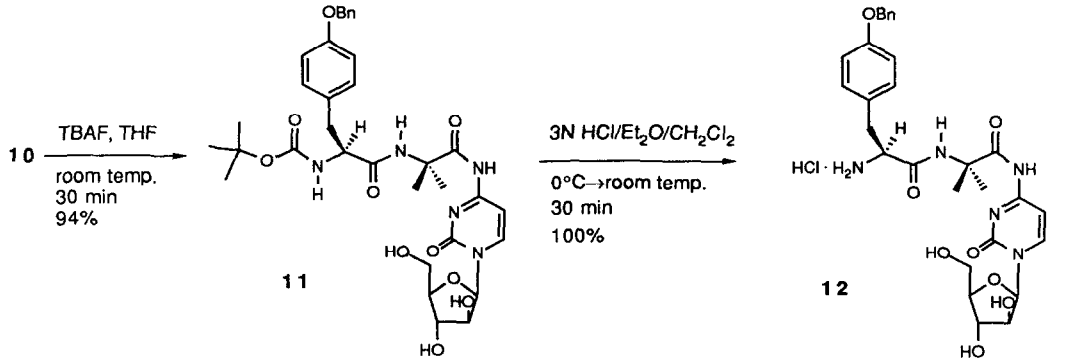
Scheme 2.



Condensation of the lithium salt of **5** with the oxazolinone **9**, prepared in 93% yield by treatment of dipeptide acid BOC-Tyr(OBn)-Aib-OH with 1 equivalent of DCC in acetonitrile,[35] led, after stirring for 16 h at -78°C→ room temp. in a THF solution, to acylation product **10** in 71% yield after column chromatography (Scheme 2). Dipeptide **8** was obtained by addition of methyl ester **7** to a THF solution of the symmetrical anhydride of *tert*-butyloxycarbonyl(*O*-benzyl)tyrosine (**6**).

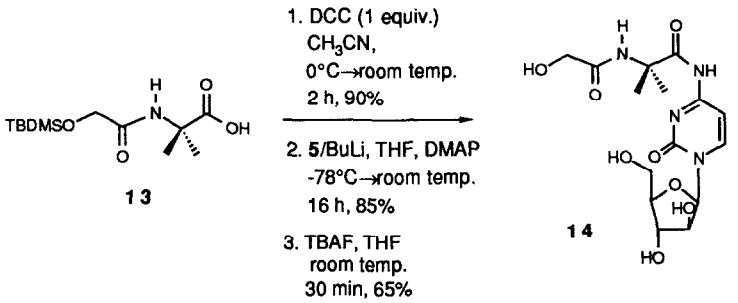
For the removal of protective groups, peptide **10** was first treated with 5 equivalents of TBAF in THF (Scheme 3). In order to achieve best yields, triol **11** was subsequently purified by column chromatography on silica gel with MeOH/$CHCl_3$ (1:5.5), dissolved in CH_2Cl_2, and added to a 3N solution of HCl (gas) in ether. After removal of the solvents and crystallization of the residual oil, hygroscopic hydrochloride **12** was obtained in 94% yield from **10**.

Scheme 3.



In a similar fashion, condensation of the lithium salt of **5** and the oxazoline derived from acid **13** led, after desilylation, to glycolate derivative **14** in 50% overall yield (Scheme 4).

Scheme 4.



With prodrug derivatives N^4-(*O*-benzyl)tyrosyl- and hydroxyacetyl-2-methylalanyl-*ara*-C (**12** and **14**), the rate of release of *ara*-C is determined by the rate of intramolecular cyclization to heterocycles **15** and **16** (Scheme 5). The half-life $t_{1/2}$ for the cyclization process is dependant on the pH of the reaction medium and was determined by ^1H NMR studies in CD_3OD in the presence of an excess of sodium acetate or acetic acid (Figure 1).

A half-life of 42 h at room temperature was found for the disappearance of dipeptidyl derivative **12** and the formation of both *ara*-C (**1**) and diketopiperazine *cyclo*(Tyr(OBn)Aib) (**15**) in CD_3OD. In the presence of NaOAc, this process was greatly accelerated ($t_{1/2}$ = 0.3 h), presumably partly due to an increase in the concentration of free amine in the reaction medium. Additionally, acetate can also directly participate and assist in the ring closure.

A similar enhancement of drug release in the presence of an excess of NaOAc was detected with hydroxy derivative **14**, where slower intramolecular lactonization led to the formation of *ara*-C and heterocycle **16** with $t_{1/2}$ = 360 h (no additive) and 1.4 h (NaOAc).

With amine **12**, a slight acceleration of drug release was observed in the presence of an excess of HOAc ($t_{1/2}$ = 31 h). Alcohol **14**, in contrast, cyclized significantly more slowly in acidic medium ($t_{1/2}$ = 860 h). This rather surprising effect could be the result of a shift in the rate-limiting step of the cyclization process to *N,O*-heterocycle **16** in acidic medium.[36]

Scheme 5.

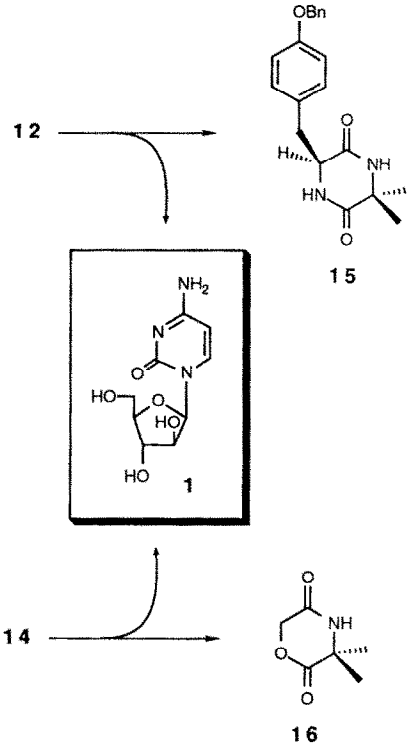
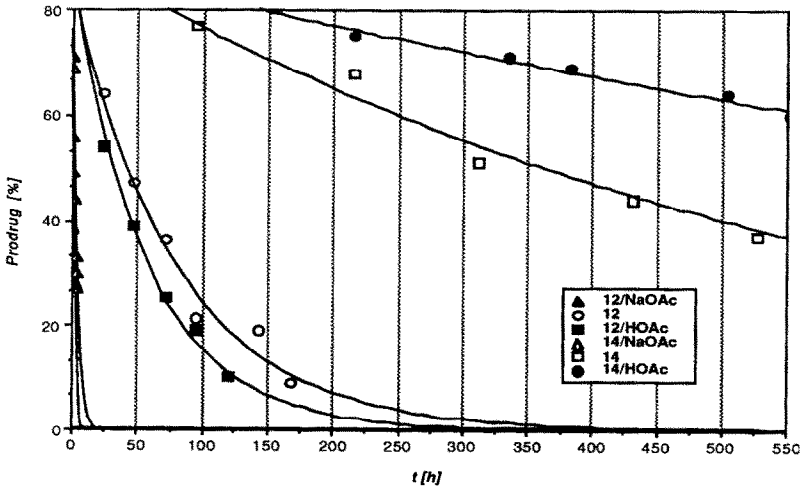


Figure 1. Cyclization of Peptidyl Prodrugs and Release of *Ara*-C as Monitored by ^1H NMR of a *ca.* $3x10^{-3}$ M Solution of **12** and **14** in CD_3OD at 22°C.[37]



	$t_{1/2}$ [h]
12/NaOAc	0.3
12	42
12/HOAc	31
14/NaOAc	1.4
14	360
14/HOAc	860

The lability of derivatives **12** and **14** in solution stands in remarkable contrast to the stability of solid material at room temperature,[38] and suggests that indeed these peptidyl derivatives are controllable slow-release forms prone to chemoreversible activation.

In conclusion, our findings indicate that especially with non-proteinogenic amino acid building blocks, the use of peptidyl derivatives of active but otherwise poorly bioavailable drugs offers an attractive alternative to standard prodrug protocols.[39] The approach described in this paper demonstrates an intramolecular cyclization of (*O*-benzyl)tyrosyl-2-methylalanyl- and glycolyl-2-methylalanyl-*ara*-C derivatives to heterocycles and *ara*-C with half-lifes between 0.3 and 860 h. No enzymatic intervention in the prodrug cleavage is required, and the regenerated *ara*-C has full biological activity *in vitro*.[40] By appropriate structural modification of the peptidyl moiety, a straightforward adoption to specific delivery requirements appears possible.[41] Further applications will be reported in due course.[42]

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37. Cyclization of **12** and **14** led to the disappearance of characteristic signals of the prodrugs and the appearance of resonances of *ara*-C and the heterocycles **15** and **16**. The half-time was determined by integration of the spectra collected in appropriate time-intervals. Cyclic dipeptide **15** was prepared independently by thermal cyclization of (*O*-benzyl)tyrosyl-2-methylalanine methyl ester hydrochloride, and was unambiguously identified as the second cyclization product besides *ara*-C. Sodium acetate (2 mg/mL) and acetic acid (2 μL/mL) were added neat to the NMR samples.
38. No decomposition product of solid **12** or **14** could be detected by NMR after 8 weeks of storage at room temperature.
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41. In order to compare the lipophilicity properties of the peptidyl prodrugs with *ara*-C, the partition coefficients of **1**, **12**, and **14** were determined in water/chloroform mixtures: $P_{ara\text{-}C\ (1)} = 0.3$; $P_{12} = 1.8$; $P_{14} = 1.2$.
42. All the compounds prepared exhibited satisfactory spectral and analytical properties. Yields refer to material of >95% purity.