Ynamines Derived from Nucleic Acids Bases: Synthesis, Reactivity and Biological Activity

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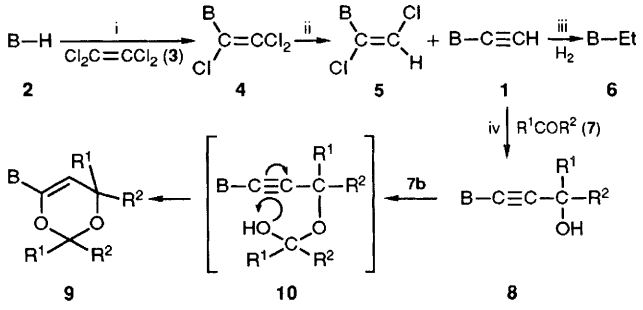
Ynamines **1a–d** are prepared by alkylation of nucleic acids bases with tetrachloroethylene followed by elimination of halogens from the intermediates **4a–d**; reaction of **1a** with acetone and cyclohexanone gives carbinols **8a** and **b** and in the case of **8b** cyclic ketal **9a** is also obtained; compound **1a** is a substrate for adenosine deaminase.

Ynamines have received only scant attention probably because of their limited stability.[1,2] Thus, ynamines derived from aromatic heterocycles and comprising a terminal acetylene moiety have not been reported to the best of our knowledge. Compounds **1** having a nucleic acid base or related moiety attached directly to a reactive but relatively small ethynyl residue are of distinct biological interest as simple nucleoside analogues. In addition, a weaker basicity of such ynamines should enhance their stability, especially toward hydrolysis. Also, compounds **1** could serve possibly as starting materials for unsaturated acyclic nucleoside analogues (see, *e.g.* refs. 3 and 4).

We now report a general synthesis as well as some chemical and biological properties of ynamines **1a–d**. The sodium salts generated *in situ* from heterocycles **2a–d** by using NaH (2 equiv.) in hexamethylphosphoric triamide (HMPA) at 60 °C were alkylated with tetrachloroethylene (**3**, 3–6 equiv.) for 15 h (Scheme 1). Trichloroenamines **4a–d** were isolated by flash chromatography on silica gel in 25–30% yields.† The alkylation is highly regioselective giving predominantly N^9-alkylated purines **4a**, **d** and N^1-substituted pyrimidines **4b**, **c**. In the instance of adenine derivatives **4a** and **d** only *ca.* 5% of N^7-isomers were present. The solvent (HMPA) is impor-

† All new compounds were characterized by elemental analyses, IR spectra and, with the exception of **1c**, by UV, NMR and mass spectroscopy.



1, **2** and **4–6a**; B = adenin-N^9-yl
b; B = thymin-N^1-yl
c; B = cytosin-N^1-yl
d; B = 2-aminoadenin-N^9-yl
7–10a; B = adenin-N^9-yl, $R^1 = R^2$ = Me
b; B = adenin-N^9-yl, $R^1 + R^2 = (CH_2)_5$

Scheme 1 *Reagents and conditions*: i, NaH, HMPA, 60 °C; ii, LiBu, THF, −70 °C; iii, Pd/C, EtOH; iv, $NaNH_2$, THF

tant for successful alkylation. Thus, alkylation of thymine **2b** with tetrachloroethylene **3** in dimethyl sulfoxide (DMSO) led only to a reduction product of **4b**, the dichlorovinyl derivative **5b** in 20% yield.

Compounds **4a–d** were converted to ynamines **1a–d** using 1-butyllithium (LiBu, 4 equiv.) in tetrahydrofuran (THF) at −70 °C for 1–3 h in 50% yields. The reaction is accompanied by a regioselective reduction of one chlorine atom of the starting materials **4a**, **c**, **d** but not **4b**. Thus, compound **5a** was obtained in 10% yield along with ynamine **1a** and it was identical with a product of alkylation of adenine **2a** with trichloroethylene. Catalytic hydrogenation of **1a** and **c** gave N^9-ethyladenine **6a** and N^1-ethylthymine **6b** with properties identical to those of authentic samples.[5,6] Unlike ynamines derived from strong tertiary bases[1] compounds **1a**, **b** and **d** are stable in aqueous solutions. Ynamine **1c** is of limited stability although it gave a correct elemental analysis and IR spectrum (KBr). The ^1H and ^{13}C NMR spectra in $(CD_3)_2SO$ at an ambient temperature and $(CD_3)_2NCDO$ at −20 °C showed, in addition to signals of **1c**, several peaks in the alkenic region indicating the presence of polyacetylene polymer(s).[7] The limited stability of **1c** cannot be attributed solely to the basicity of the cytosine moiety because ynamine **1d** containing a more basic 2-aminoadenine residue is quite stable.

The reaction of ynamine **1a** with acetone **7a** in the presence of $NaNH_2$ in THF gave the carbinol **8a** in 45% yield. The higher yield (70%) was obtained with cyclohexanone **7b** but compound **8b** was accompanied by cyclic ketal **9a** (30%). The latter became virtually the sole product when more than 2 equiv. of **7b** were employed. Obviously, carbinol **8b** is an intermediate in the process which includes formation of hemiketal **10a** and subsequent cyclization to give **9a**. It is noteworthy that a base-catalysed reaction of diacetylene carbinols with formaldehyde was reported[8] to give dioxolane derivatives also *via* the corresponding hemiacetals.

Ynamine **1a** was deaminated with adenosine deaminase (ADA) under standard conditions.[9] After 3 days *ca.* 90% deamination was observed at room temperature as shown by TLC in CH_2Cl_2–MeOH (9 : 1) and UV spectra (Fig. 1). The N^9-(1-propyn-3-yl)- and N^9-vinyladenine as well as compound **1d** were inert towards ADA. Compound **1a** is the simplest substituted adenine amenable to deamination catalysed with ADA. Compounds **4b**, **1a**, **d** and **8b** inhibited the growth of murine leukaemia L 1210 cells with IC_{50} 40, 100, 125 and 150 μmol dm^{-3}, respectively, as determined by a clonogenic assay.[10] Compounds **1a**, **d** and **4a** suppressed the tumour growth in cultures of mouse colon tumour C38 and human lung tumour H8 or H116 at 0.04, 0.5 and 1 mg per disk,

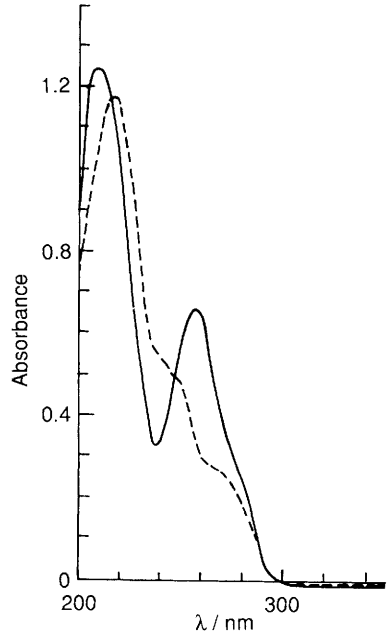


Fig. 1 Deamination of ynamine **1a** with ADA. The incubation mixture contained 4.7 μmol **1a** ml^{-1} and 0.66 enzyme unit ml^{-1} in 0.05 mol dm^{-3} Na_2HPO_4, pH 7.5. An aliquot was diluted with buffer and the UV spectrum was recorded. (———) UV spectrum before addition of ADA, (– – – –) after 3 days of incubation of **1a** with ADA at room temperature.

respectively, as shown by a zone assay.[10] Further investigation of the synthetic utility of ynamines **1a–d** as well as other biological tests are in progress.

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