Synthesis by conjugate radical addition of new heterocyclic amino acids with nucleic acid bases in their side chains

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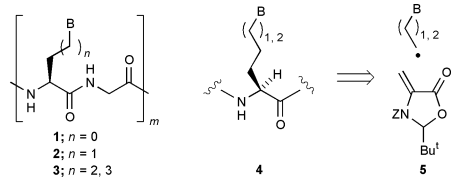
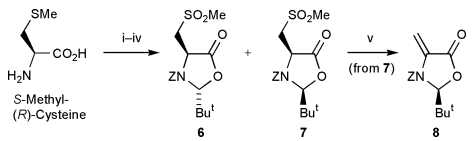
_N_-(2-Iodoethyl) and _N_-(3-iodopropyl)pyrimidines and purines undergo stereoselective conjugate radical addition with an optically active oxazolidinone acceptor to give _syn_-adducts that can be converted into pyrimidine and purine amino acids.

Peptide-based nucleic acid analogues (PNAs) have attracted much attention as molecules with the potential to interact with nucleic acid chains.[1] Suggested applications include antisense properties.[2] Nielsen's PNA has been shown to form duplexes with the complementary DNAs.[1] DNA recognition using analogues with a 'real' peptide backbone has, however, proved more elusive. Substituted alanine oligomers **1** (B = pyrimidine or purine base) and homologues **2** (termed α-PNA[3]) do not demonstrate hybridisation with DNA and insufficient flexibility of the polypeptide chain has been suggested as the cause,[4] whereas triplex formation between tetrapeptides of type **2** and poly(dT) or poly(dU) has been reported.[5] Our interest in unusual amino acids led us to propose the homologous amino acids **4** carrying the nucleic acid bases with a 3- or 4-methylene tether to the peptide backbone, as components for PNA variant **3**. Residues **4** are also analogues of natural pyrimidine and purine amino acids.[6] We report here our flexible methodology based on stereospecific radical chemistry.[7]

In contrast to published routes to residues with C_2 tethers,[3,5,8] we determined to link preformed heterocycles with the peptide backbone by forming a *carbon–carbon* bond in the tether, and proposed to generate the C(β)–C(γ) bond by conjugate radical addition to chiral acceptor **7** (Scheme 1).[9] The (*S*)-acceptor **8** was prepared from *S*-methyl-(*R*)-cysteine (Scheme 2) by adaptation of a published sequence to the *N*-benzoyl analogue.[10] *syn*-Sulfone **7** was formed as a 10:1 mixture with its *anti*-diastereoisomer **6** [57% overall from *S*-methyl-(*R*)-cysteine] and easily separated by column chromatography.[10] The *syn*-configuration was supported *inter alia* by mutual NOE enhancements between C-2(H) and C-4(H). Base treatment afforded (*S*)-oxazolidinone **8** as a crystalline solid.



Scheme 1



Scheme 2 *Reagents*: i, NaOH aq.; Bu^{t}CHO, Dean-Stark; iii, $PhCH_2OCOCl$ (ZCl); iv, oxone®, MeCN–H_2O; v, DBU.

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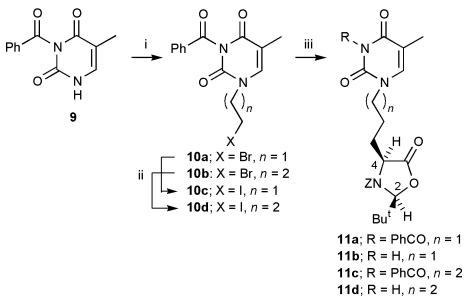
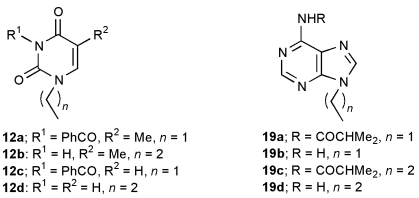
The radical precursors were haloalkyl pyrimidines and purines, prepared from the appropriately protected heterocyclic base and an ω-haloalcohol.[11] Thus 3-benzoylthymine **9**[12] was coupled with 2-bromoethanol or 3-bromopropanol (DIAD, Ph_3P) to afford the 1-(ω-bromoalkyl) derivatives **10a,b**, respectively (Scheme 3). Attempts to generate radicals from these bromides proved fruitless, so they were converted directly to the iodoalkyl compounds **10c,d**, respectively (NaI, propanone reflux; 85, 87% from **9**).

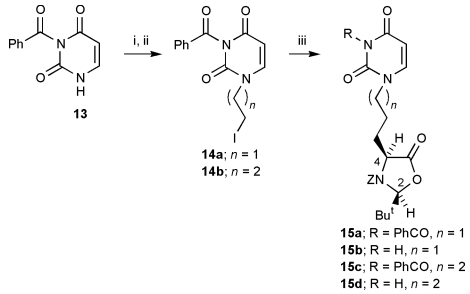


Scheme 3 *Reagents*: i, $HO(CH_2)_{n+1}Br$, $Pr^iO_2CN{=}NCO_2Pr^i$, Ph_3P; ii, NaI, Me_2CO reflux; iii, Method A: **10** (1 mol equiv.), Bu_3SnH (1 mol equiv.), AIBN (0.1 mol equiv.), toluene reflux; Method b: **8** (2 mol equiv.), Bu_3SnCl (0.3 mol equiv.), $NaBH_3CN$ (2 mol equiv.), AIBN (0.1 mol equiv.).

Iodide **10c** was treated under two protocols differing in the method for radical generation;[9] method A: with oxazolidinone **8** (1 mol equiv.) in toluene at reflux containing AIBN (0.1 mol equiv.) and dropwise addition of Bu_3SnH (1 mol equiv.); or method B: with oxazolidinone **8** (2 mol equiv.), Bu_3SnCl (0.3 mol equiv.), $NaBH_3CN$ (2 mol equiv.) and AIBN (0.1 mol equiv.) in *tert*-BuOH at reflux. Method A afforded the conjugate addition product **11a** (26%) and reduction product **12a** (24%), whereas method B after 16 h afforded 54% of conjugate addition products consisting of the adduct **11a** (24%) and the 3-debenzoylated derivative **11b** (30%), with no reduced material. The extent of debenzoylation was time dependent; a reaction time of 40 h led to **11b** as the sole addition product (47%). This suggests the deacylation may be *via* hydride-mediated reduction of the out-of-plane benzoyl carbonyl group, a possibility supported by an observed decrease in debenzoylation when less $NaBH_3CN$ is used in method B, and that debenzoylation of **10** occurs in the presence of $NaBH_3CN$ alone.[13] When 1-iodopropylthymine derivative **10d** was treated under method B, adduct **11c** was not found and deacylated adduct **11d** was isolated (25%) along with reduction product **12b** (75%).

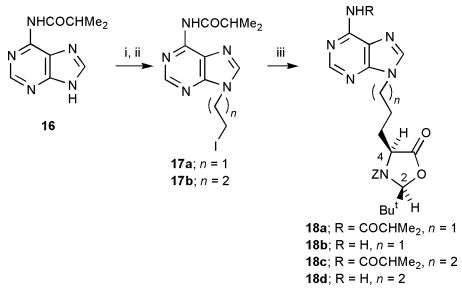
We elected to extend these standard protocols (method B preferred) to other pyrimidines and purines rather than optimise each conjugate addition. Thus 3-benzoyluracil **13**[12] was



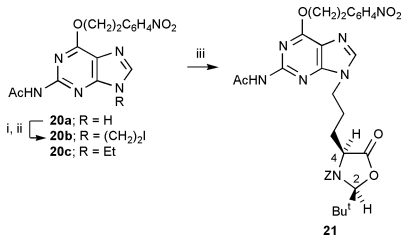


Scheme 4 *Reagents*: i, ii as Scheme 3; iii, Method B.

converted into the 1-(iodoalkyl) derivatives **14a,b** (Scheme 4). Method B applied to **14a** afforded addition product **15a** (41%) and reduction product **12c** (46%); when the reaction was left for 2 days, deacylated addition product **15b** (51%) was isolated. Homologue **14b** gave debenzoylated adduct **15d** (44%) with reduced material **12d** (51%).‡ In the purine series, the 9-(iodoalkyl)adenines **17a,b** were prepared from (2-methylpropionyl)adenine **16**[14] (Scheme 5). Using method B, iodoethyl compound **17a** led to the expected mixture of conjugate addition [40%; acylated **18a** (26%) and deacylated **18b** (14%)] and reduction [36%; acylated **19a** (17%) and deacylated **19b** (19%)]. Iodopropyl derivative **17b** likewise gave adducts [22%; acylated **18c** (12%) and deacylated **18d** (10%)] and reduced compounds [34%; acylated **19c** (11%) and deacylated **19d** (23%)]. Finally, a protected guanine **20a**[15] was converted into the 9-iodoethyl derivative **20b** (Scheme 6) and method A led to adduct **21** (21%) and reduction to **20c** (20%).

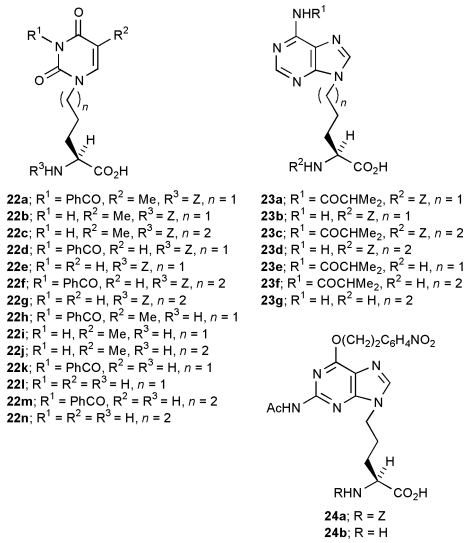


Scheme 5 *Reagents*: i, ii as Scheme 3; iii, Method B.



Scheme 6 *Reagents*: i, ii as Scheme 3; iii, Method A.

The illustrated conjugate radical addition products were all *syn*-adducts, as determined by NOE studies [enhancements between C-2(H) and C-4(H)]. Only one diastereoisomer was visible in the ^{1}H NMR spectra at 300 MHz. All of these *syn*-oxazolidinones could be easily and efficiently converted into *N*-benzyloxycarbonyl-(*S*)-amino acids (suitable for peptide coupling) by base hydrolysis (LiOH, aq. THF, 0 °C, 30–60 min; 70–98%). Thus the three thymine-substituted Z-amino acids **22a–c** (having 3- or 4-carbon tethers for the pyrimidine) were prepared from the adducts **11a**,**b**,**d**, respectively. The uracil Z-amino acids **22d–g** were likewise prepared from adducts **15a–d**, respectively, as were adenine derivatives **23a–d** (from **18a–d**, respectively) and guanine Z-amino acid **24a** (from **21**). To monitor optical purity, the Z group was removed by hydrogenolysis (Pd–C, EtOH–H_2O; 60–80%) to afford the amino



acids **22h–n**, **23e–g** and **24b**, analysed by esterification (AcCl, EtOH, reflux) and subsequent conversion to the Mosher amides (*R*-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride, pyridine);[16] ^{19}F NMR spectroscopy revealed, *e.g.* 86–91% e.e. for the amino acids **22i**,**j**,**l**,**m**, and **23f**.

We have thus made available a range of novel pyrimidinyl and purinyl amino acids for application, for example, in PNA variants.

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Notes and references

‡ The yield of **15d** could be increased to 62% by using 5 mol equiv. of acceptor **8** in method B, but we more usually used 2 mol equiv. of this valuable optically active intermediate. When less than 2 mol equiv. $NaBH_3CN$ was used, some of the benzoylated adduct **15c** was isolated.

1 For recent reviews, see: B. Hyrup and P. E. Nielsen, *Bioorg. Med. Chem.*, 1996, **4**, 5; P. E. Nielsen and G. Haaima, *Chem. Soc. Rev.*, 1997, **26**, 73.
2 H. K. Larsen, T. Bentin and P. E. Nielsen, *Biochim. Biophys. Acta*, 1999, **1489**, 159.
3 N. M. Howarth and L. P. G. Wakelin, *J. Org. Chem.*, 1997, **62**, 5441.
4 See: M. Kuwahara, M. Arimitsu and M. Sisido, *Tetrahedron*, 1999, **55**, 10 067, and refs. therein.
5 T. Yamakazi, K. Komatsu, H. Umemiya, Y. Hashimoto, K. Shudo and H. Kagechika, *Tetrahedron Lett.*, 1997, **38**, 8363.
6 See, for example: R. M. Adlington, J. E. Baldwin, D. Catterick and G. J. Pritchard, *J. Chem. Soc., Perkin Trans. 1*, 1999, 855.
7 See: C. J. Easton, *Chem. Rev.*, 1997, **97**, 53, for a review of radical reactions in amino acid synthesis.
8 A. Lenzi, G. Reginato and M. Taddei, *Tetrahedron Lett.*, 1995, **36**, 1713.
9 J. R. Axon and A. L. J. Beckwith, *J. Chem. Soc., Chem. Commun.*, 1995, 549, and refs. therein.
10 S. G. Pyne, B. Dikic, P. A. Gordon, B. W. Skelton and A. H. White, *Aust. J. Chem.*, 1993, **46**, 73.
11 *Cf.* G. Cadet, C.-S. Chan, R. Y. Daniel, C. P. Davis, D. Guiadeen, G. Rodriguez, T. Thomas, S. Walcott and P. Scheiner, *J. Org. Chem.*, 1998, **63**, 4574.
12 K. A. Cruickshank, J. Jiricny and C. B. Reese, *Tetrahedron Lett.*, 1984, **25**, 681.
13 *Cf.* S. Kim, T. A. Lee and Y. Song, *Synlett*, 1998, 471, for radical addition to imides.
14 J. Zhou, K. Bouhadir, T. R. Webb and P. B. Shevlin, *Tetrahedron Lett.*, 1997, **38**, 4037.
15 J. Zhou, J.-Y. Tsai, K. Bouhadir and P. B. Shevlin, *Synth. Commun.*, 1999, **29**, 3003.
16 J. A. Dale, D. L. Dull and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2543.