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3-(Aminomethyl)-2-(carboxymethyl)isoxazolidinyl nucleosides: building blocks for peptide nucleic acid analogues

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Abstract—The synthesis of orthogonally protected 3-(aminomethyl)-2-(carboxymethyl)isoxazolidinyl thymine, a convenient monomer for the preparation of novel isoxazolidinyl peptide nucleic acid analogues, has been achieved through enantioselective 1,3-dipolar cycloaddition between *N*-glycosyl nitrones and vinyl acetate.

1. Introduction

Peptide nucleic acid **1** is an excellent mimic of nucleic acids in which the sugar-phosphate backbone is replaced by a pseudopeptide backbone composed of *N*-(2-amino-methyl) glycine units.[1] PNA **1** binds complementary DNA and RNA sequences with a much stronger affinity and with more stable binding than the corresponding naturally occurring complementary nucleic acids.[2] This unique feature presents PNA and its analogues as drug candidates for the treatment of cancer and viral infections (Chart 1).[3]

The nucleic acid binding properties of PNA have also been exploited to obtain powerful biomolecular tools, such as antisense probes and biosensors with immediate applications in medicine.[4] The hybridization properties of **1** to form PNA/DNA and PNA/RNA duplexes can be improved upon by using more rigid PNA analogues such as **2** in which the aminomethylglycine unit and the methylenecarbonyl linker are connected by a methylene group.[5] In order to increase the water solubility of PNAs by means of protonation, the pyrrolidine analogues **3** were prepared.[6] Similarly, other pyrrolidine-based PNA analogues with different connectivities between the base moiety and the pseudopeptide backbone have gained prominence during recent years.[7]

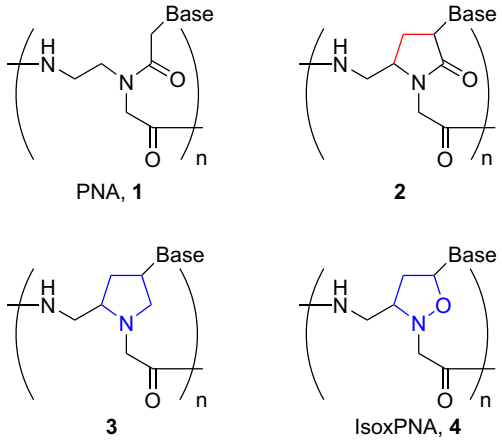

Chart 1. Peptide nucleic acid analogues.

We have recently reported on our successful efforts to develop general routes to isoxazolidinyl nucleosides,[8] a new class of nucleoside analogues in which the furanose ring has been replaced by an isoxazolidine ring.[9] Our synthetic strategy may now be applicable to the synthesis of suitable monomers for preparing the hitherto unknown isoxazolidinyl analogues of PNA **4**. Compounds **4** can also be considered as conformationally restricted PNA analogues in which it is expected that the endocyclic oxygen atom will decrease the basicity of the heterocyclic ring, thus

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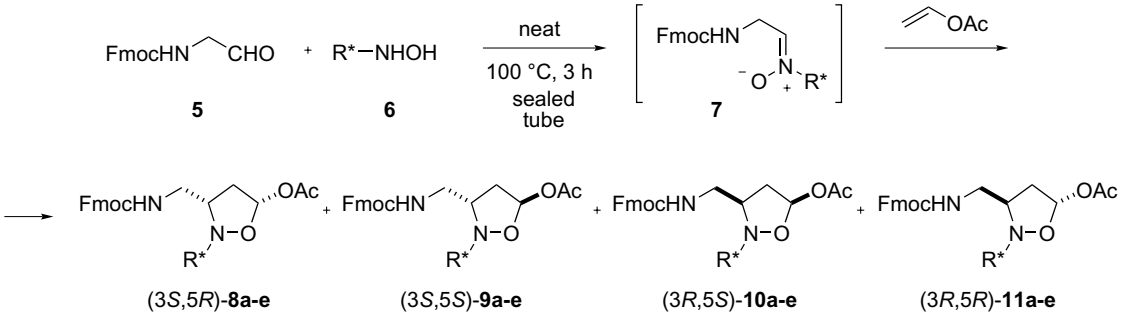
maintaining good hydrophilicity. The hydrophilicity[10] of analogues **4** ($c\log P = -2.73$) is closer to **2** ($c\log P = -3.12$) than to **3** ($c\log P = -1.95$) while the basicity of the isozazolidine ring is lower than that of the pyrrolidine ring.

Herein, we report our initial efforts directed towards the preparation of a enantiomerically pure monomer suitable of being used for the preparation of oligomers **4**.

2. Results and discussion

Our approach is based on the construction of the isoxazolidine ring in a 1,3-dipolar cycloaddition reaction of nitrone **7** and vinyl acetate. Compound **7** was formed in situ from Fmoc-glycinal **5**, obtained from commercially available Fmoc glycine by reduction of the corresponding acid chloride with Bu_3SnH in the presence of $Pd(PPh_3)_4$,[11] and sugar-hydroxylamines **6**, prepared from the parent free anomeric sugar by treatment with hydroxylamine hydrochloride[12] (Scheme 1, Table 1).

Chiral hydroxylamines **6b–e** served as chiral auxiliaries for the present study, while hydroxylamine **6a** was used for the purpose of comparison. The reaction with the achiral hydroxylamine **6a**[13] (Table 1, entry 1) afforded a 1.5:1 mixture of *cis*:*trans* 3,5-adducts showing a preference for an *exo* attack as expected for an inverse demand cycloaddition reaction. The regiochemistry of the reaction was also in



Scheme 1. Enantioselective 1,3-dipolar cycloaddition.

Table 1. Synthesis of isoxazolidines **8**–**11**[a]

Entry	6	R*–NHOH	Yield[b] (%)	8:9:10:11[c]	*cis*:*trans*[d]	*Si*:*Re*[e]
1	**a**	HOHN, $CO_2{}^tBu$	60	1.5:1:—:—	60:40	—
2	**b**	OBn, BnO, BnO, OBn, NHOH	63	42:26:32:0	74:26	68:32
3	**c**	$^tBuMe_2SiO$, NHOH	90	43:41:16:0	59:41	84:16
4	**d**	$^tBuPh_2SiO$, NHOH	92	49:32:19:0	68:32	81:19
5	**e**	NHOH	91	72:16:12:0	84:16	88:12

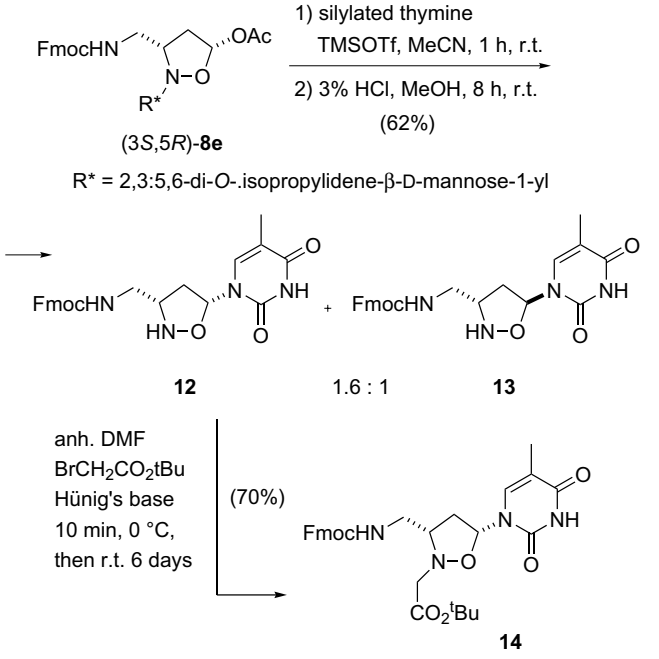
[a] The reaction was carried out without solvent over 3 h at 100 °C and using 30 equiv of vinyl acetate, 1.0 equiv of aldehyde and 1.2 equiv of hydroxylamine.
[b] Isolated yield of the mixture of diastereoisomers.
[c] Calculated from the NMR of the crude mixture.
[d] Refers to the relative configuration of 3- and 5-substituents, indicating the *exo*/*endo* selectivity.
[e] Refers to the diastereotopic faces of the nitrone, indicating the diastereofacial selectivity.

agreement with that expected for those reactions. When D-glucose derived chiral hydroxylamine **6b** was used, a moderate *exo* selectivity and poor diastereofacial differentiation was observed (Table 1, entry 2). The best results were observed when five membered sugar derived hydroxylamines were employed as chiral auxiliaries. Thus, by using D-ribose hydroxylamine **6c**, a good diastereofacial induction (84:16) was observed (Table 1, entry 3); however, the reaction did not show any *exo*/*endo* selectivity. By changing the protecting group at the primary hydroxyl to a bulkier *tert*-butyldiphenyl group similar results were obtained although the reaction showed to be slightly more *exo* selective (Table 1, entry 4). Finally, the use of D-mannose derived hydroxylamine **6e** led to the best results (Table 1, entry 5) affording good *exo* and diastereofacial selectivities. Thus, the corresponding adducts **8e**–**10e** were separated by semipreparative HPLC and completely characterized.[14] The relative configuration of the cycloadducts obtained was ascertained by conventional NMR techniques including 2D NOESY, COSY and HMBC experiments.

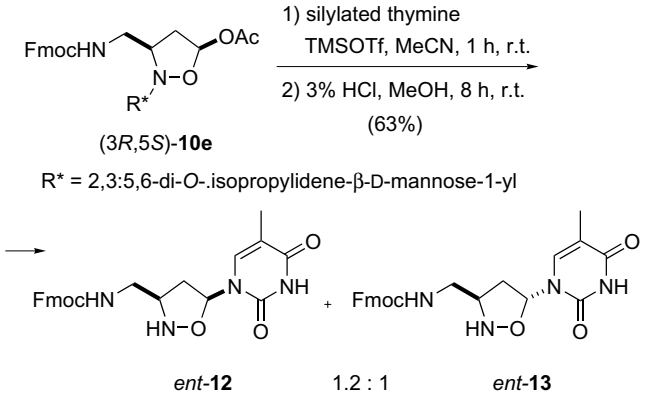
On the other hand, the absolute configuration was tentatively assigned on the basis of similar results previously obtained with *N*-glycosyl nitrones by us[15] and others[16] in dipolar cycloaddition reactions. According to these studies, the less hindered *Si*-face of the in situ formed nitrone is favoured towards an *exo* attack leading preferentially to the (3*S*,5*R*) adducts **9**. As a general trend, the reaction illustrated in Scheme 1 showed a moderate *exo*/*endo* (*cis*/*trans*) selectivity and good diastereofacial selectivity when 5-membered glycosyl units were used as chiral auxiliaries.

The N-glycosylation of pure **8e** with silylated thymine, following the Vörbruggen protocol,[17] and subsequent acidic treatment (3% HCl in EtOH) to eliminate the chiral auxiliary furnished a 1.6:1 mixture of *cis*- and *trans*-isoxazolidinyl nucleosides **12** and **13**, respectively (Scheme 2). After purification by MPLC (EtOAc/MeOH, 98:2, 20 bar) compounds **12** {$[\alpha]_D = +4$ (*c* 0.82, $CHCl_3$); mp 151–154 °C} and **13** {$[\alpha]_D = +8$ (*c* 1.02, $CHCl_3$); mp 154–156 °C} were isolated. The minor adducts **9e** and **10e** were also submitted to the protocol illustrated in Scheme 2. Thus, when the *trans* adduct **9e** was submitted to the same reaction sequence (N-glycosylation and acidic treatment), an identical result to that observed for **8e** was obtained as expected for a typical glycosylation reaction. Similarly, the treatment of pure **10e** under the same conditions as above afforded a 1.2:1 mixture of *ent*-**12** and *ent*-**13**, whose physical and spectroscopic properties were identical to those of **12** and **13** except for the sign of the specific rotation (Scheme 3). From a synthetic point of view, it would be more advisable to use a mixture of **8e** and **9e** since the same result is obtained for each separated compound in the glycosylation reaction.

Treatment of pure **12** with *tert*-butyl 2-bromoacetate in anhydrous DMF in the presence of $^{i}PrEt_2N$ afforded **14** in 70% isolated yield after purification by radial chromatography.[18] Compound **14**, which has been prepared on a scale of 300–800 mg, is easy to manipulate since it is orthogonally protected, which should be taken into consideration for further use in the solid-phase synthesis of PNA-oligomers, for which basic-sensitive Fmoc protecting group is particularly advisable. The acid-sensitive *tert*-butyl ester will allow peptide synthesis by means of its chemoselective hydrolysis. The synthesis of other (aminomethyl)isoxazolidinyl nucleosides with different heterocyclic bases and their use for preparing isoxazolidinyl PNA is currently under investigation.



Scheme 2. Synthesis of 3-(aminomethyl)-2-(carboxymethyl)isoxazolidinyl thymidine **14**.



Scheme 3. Synthesis of isoxazolidinyl nucleosides *ent*-**12** and *ent*-**13**.

Acknowledgements

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14. All new compounds exhibited consistent spectral and microanalytical data. Data for **8e**: $[\alpha]_D^{20} = +50$ (*c* 1.28, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 1.27 (s, 3H), 1.30 (s, 3H), 1.38 (s, 3H), 1.42 (s, 3H), 1.97 (s, 3H), 2.04 (br d, 1H, $J = 12.6$ Hz), 2.57–2.66 (br ddd, 1H, $J = 6.6$, 7.6, 12.4 Hz), 3.31 (m, 2H), 3.59 (m, 1H), 3.91–3.96 (m, 1H), 3.97–4.03 (m, 2H), 4.10–4.15 (m, 1H), 4.21–4.26 (m, 1H), 4.61 (br s, 2H), 4.67 (br s, 1H), 4.75–4.81 (m, 1H), 4.94 (d, 1H, $J = 5.6$ Hz), 5.13 (br t, 1H, $J = 6.3$ Hz), 6.27 (d, 1H, $J = 6.1$ Hz), 7.25–7.70 (m, 8H). Data for **9e**: $[\alpha]_D^{20} = +38$ (*c* 0.22, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 1.25 (s, 6H), 1.31 (s, 3H), 1.39 (s, 3H), 2.02 (s, 3H), 2.19 (br td, 1H, $J = 5.8$, 13.9 Hz), 2.42 (br td, 1H, $J = 7.5$, 14.1 Hz), 3.09–3.18 (m, 1H), 3.19–3.27 (m, 1H), 3.63–3.72 (m, 1H), 3.90–3.97 (m, 1H), 4.16 (t, 1H, $J = 6.6$ Hz), 4.25–4.36 (m, 3H), 4.34–4.39 (m, 1H), 4.61 (br s, 2H), 4.69 (br s, 1H), 4.84 (d, 1H, $J = 6.1$ Hz), 5.10 (br t, 1H, $J = 5.8$ Hz), 6.31 (br d, 1H, $J = 5.8$ Hz), 7.24–7.70 (m, 8H). Data for **10e**: $[\alpha]_D^{20} = -2$ (*c* 0.38, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 1.26 (s, 3H), 1.32 (s, 3H), 1.40 (s, 3H), 1.42 (s, 3H), 1.98 (s, 3H), 2.07 (br d, 1H, $J = 13.9$ Hz), 2.56 (br ddd, 1H, $J = 6.3$, 8.6, 14.4 Hz), 3.25–3.34 (m, 1H), 3.34–3.43 (m, 1H), 3.52–3.63 (m, 1H), 4.21–4.31 (m, 3H), 4.34 (m, 1H), 4.39–4.44 (m, 1H), 4.61 (br s, 2H), 4.68 (s, 1H), 4.73–4.77 (m, 1H), 4.87 (d, 1H, $J = 5.3$ Hz), 5.18 (br t, 1H, $J = 6.0$ Hz), 6.37 (d, 1H, $J = 5.8$ Hz), 7.24–7.70 (m, 8H).
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18. Data for **14**: $[\alpha]_D^{20} = +3$ (*c* 0.80, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 1.52 (br s, 9H), 1.97 (s, 3H), 2.20 (br s, 1H), 2.97–3.14 (m, 2H), 3.22–3.32 (m, 1H), 3.47 (br s, 1H), 3.54 (br d, 1H, $J = 16.0$ Hz), 3.74 (br d, 1H, $J = 15.2$ Hz), 4.21 (t, 1H, $J = 5.9$ Hz), 4.46 (br d, 2H, $J = 5.9$ Hz), 5.34 (br s, 1H), 6.00 (br s, 1H), 7.32–7.70 (m, 8H), 8.12 (br s, 1H), 8.73 (br s, 1H).