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Towards novel amide-modified oligonucleotides: asymmetric synthesis of 5′-(*S*)-methyl-3′-carboxymethylene-3′-deoxythymidine

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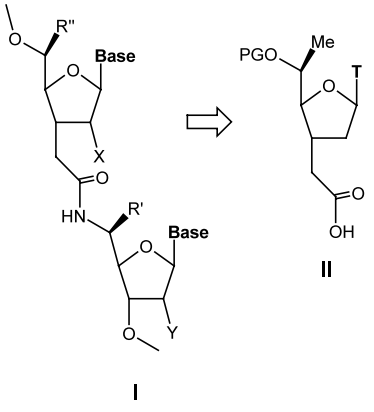
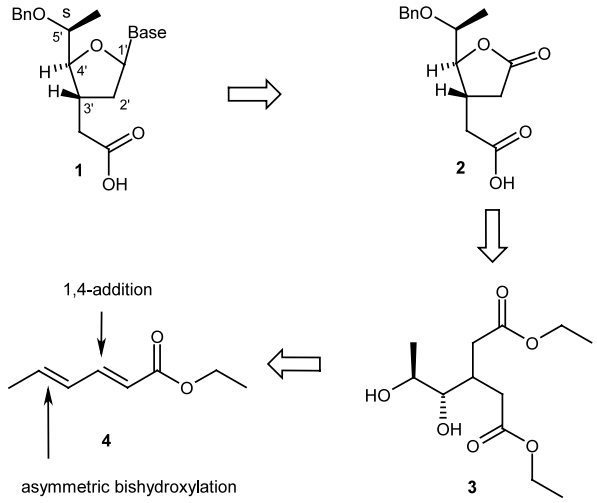
Abstract—The synthesis of novel 5′-(*S*)-methyl-3′-carboxymethylene-3′-deoxythymidine is reported. Key steps involve diastereoselective lactonization, enantioselective enzymatic ester hydrolysis and diastereoselective glycosidation of a key intermediate with thymine with 100% β-selectivity via Lewis acid mediated cleavage of a [3.2.1] oxabicyclic lactone.

Previously we have reported that the replacement of the phosphoric acid diester in oligonucleotides with an amide **I** can lead to increased affinities towards complementary RNA and DNA targets and higher nuclease resistance. We have also demonstrated that substituents X and Y, as well as R′ (R = Me) significantly further preorganize the amide backbone and lead to substantial improvements in their binding affinities towards complementary oligonucleotide targets.[1] We were questioning whether the properties of amide containing oligonucleotides could be further improved by additional substituents (R″) in the 5′-position of the carboxylic acid portion of the amide dimer. Here, we report the stereoselective synthesis of **II** (R″ = Me) from achiral starting materials.



I II

The retrosynthetic analysis of the desired 5′-(*S*)-methyl-3′-carboxymethylene-3′-deoxythymidine is outlined in Scheme 1. We envisioned selective introduction of the nucleobase at a late stage facilitating the synthesis of all four base analogs (T, C, A, G). Therefore, a key challenge would be the selective introduction of the nucleobase from the β-face of an intermediate precursor. We anticipated that cyclizations of **3** to **2** would control the stereochemistry at the 3′ position, placing the carboxethoxymethylene group *trans* to the C(5′) group. Intermediate **3** should be readily available from sorbic acid ethyl ester.



Scheme 1. Retrosynthetic analysis.

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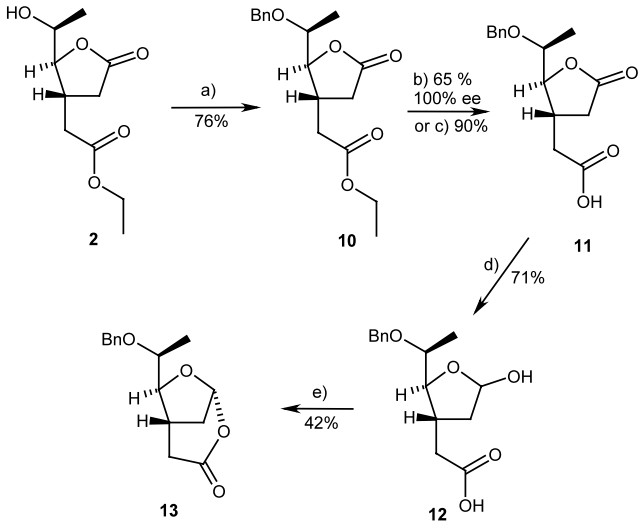
The realization of this retrosynthetic analysis is outlined in Scheme 2. Sorbic acid ethyl ester **4** was submitted to Sharpless asymmetric bishydroxylation reaction according to published procedures,[2] yielding, upon ketalization, compound **6** (45%, 80% ee), which was reacted with sodium salt of diethylmalonate to give **7**. Remarkably, **7** could not be decarboxylated under thermal conditions (NaCl, H_2O, DMSO, 160°C, 16 h)[3] but readily reacted to **8** when the reaction was conducted under microwave irradiation (NaCl, H_2O, DMSO, MW (800 W), 0.3 h). Treatment of **8** with trifluoroacetic acid in ethanol and dichloroethane resulted in acetonide cleavage and subsequent lactonization gave compounds **2** and **9** (ratio **2**:**9**: 5.5:1, ^{1}H NMR) in 42% and 11% isolated yield, respectively.[4] Our expectation that cyclization would occur with concomitant stereo-control at C(3′) was fully met. The minor product **9** presumably derives from the 3′ epimer of **2** which readily forms the bicyclic 6,5 *cis*-fused dilactone. We have demonstrated that isolated **2** does not isomerize to **9** when resubmitted to the acidic reaction conditions employed for its formation.

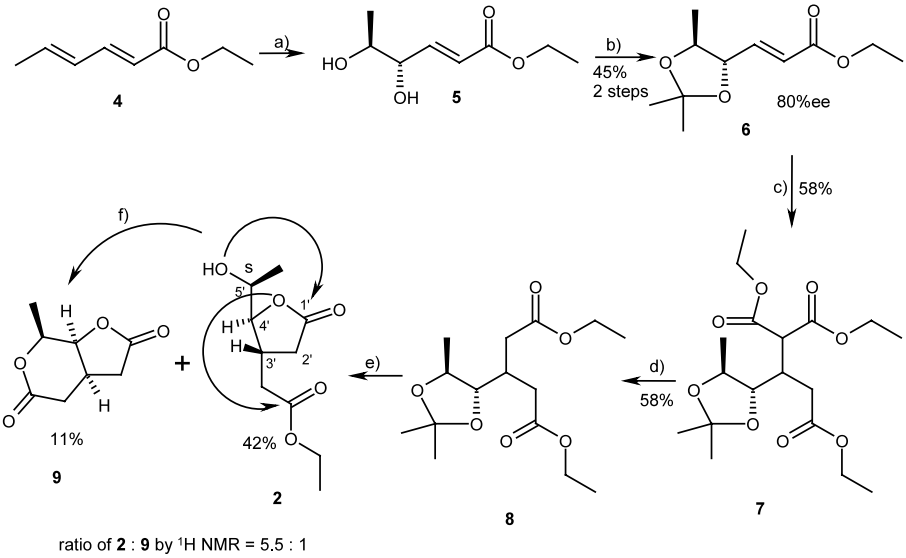
Under basic conditions, however (Scheme 2), **2** reacted to **9**, presumably through isomerization to the more stable 6-membered ring lactone by attack of the C(5′)-hydroxyl group onto the C(1′) followed by cyclization of the liberated C(4′) hydroxyl group onto the ethyl ester. Taking these results into consideration we opted to protect the 5′-alcohol using benzyl trichloroacetamide and a catalytic amount of trifluoromethanesulfonic acid by the method of Bundle to give **10** in 76% yield.[5] Attempts to reduce the lactone **10** to the corresponding lactol in the presence of the ethyl ester were unsuccessful.[6] We therefore lyophilized **10** with aqueous NaOH in THF to carboxylic acid **11**, which could be selectively reduced to the lactol by DIBAL to give **12**. The required hydrolysis of ethyl ester **10** to **11** was also seen as a welcome opportunity to increase the enantiomeric excess above the 80% ee derived from the asymmetric Sharpless bishydroxylation reaction.

Screening of a number of different hydrolases led us to select the *Bacillus licheniformis* protease Novozyme 243 from Novo, which exclusively hydrolyzed the desired enantiomer of **10** yielding **11** in 63% yield and 100% ee on multigram scale in a biphasic reaction system.[7] Treatment of crude **12** with pyridinium toluenesulfonic acid in toluene lead to bicyclic **13** (Scheme 3).

We next focused our attention on the introduction of thymine. To this end we worked with a model system



Scheme 3. (a) 2 equiv. $BnO(NH)CCl_3$, 0.1 equiv. $MeSO_3H$, cyclohexane:CH_2Cl_2 (2:1), rt, 4 h. (b) *Bacillus licheniformis* protease Novozyme 243, DIPE/K-buffer pH 6.8, 100% ee. (c) 2.4 equiv. NaOH, THF, H_2O, 0°C→rt, 1.5 h. (d) 2.2 equiv. DIBAL, Tol, −78°C, 0.5 h, 10 min, 0°C. (e) 0.1 equiv. PPTS, 3 Å MS, PhMe, 70°C.

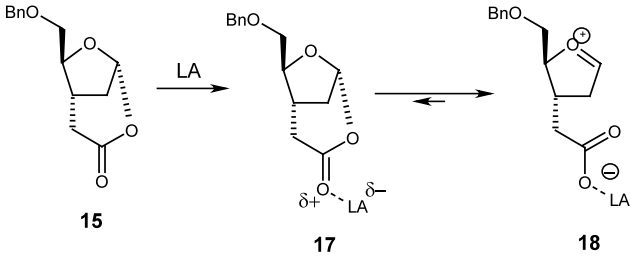


Scheme 2. (a) 1.5 equiv. α-AD-mix, 1 equiv. $MeSO_2NH_2$, ^{t}BuOH:H_2O (1:1), 4°C, 18 h. (b) $(MeO)_2CMe_2$, cat TsOH, acetone. (c) 1.1 equiv. $(EtOOC)_2CH_2$, 1.1 equiv. NaOEt, EtOH, rt, 4 h. (d) 9 equiv. H_2O, 0.5 equiv. NaCl, DMSO, microwave, 800 W, 0.25 h. (e) CF_3COOH:EtOH:CH_2Cl_2 (1:1:8). (f) NaH, THF.

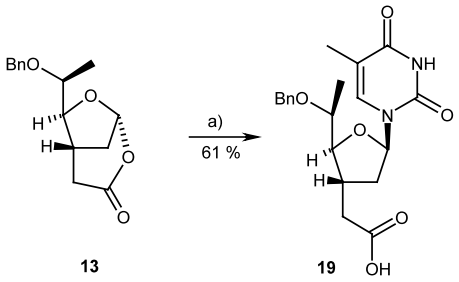
lacking the 5′(*S*) methyl group, which was readily available from previously described intermediates.[8] We envisioned two different alternatives in order to favor introduction of thymine from the β-face: Lewis acid mediated glycosidation and side group participation of the 3′-methylene carboxylic acid in **14**,[9–11] or reaction with bicyclic intermediate **15**. Reaction of bis-silylated thymine with **14** proceeded well in the presence of a number of different Lewis acids and solvents (Scheme 4, entries 1–4).[12] Unfortunately, β/α-selectivity could not be improved to a ratio of more than 3:1, and the two epimers could not be separated by chromatography. We next turned our attention to bicyclic **15** but unfortunately all commercially available Lewis acids tested gave results not superior to those obtained with **14** (data not shown). In the absence of any Lewis acid **15** did react with bis-silylated thymine, however only at elevated temperatures, and, to our disappointment with unsatisfactory β/α-selectivity (Scheme 4, entry 5).

We concluded that Lewis acid activation of **15** led to an oxonium ion which was little or not stabilized by the internal, metal-coordinated carboxylate and that the ratio of **16**β/α is controlled mainly by steric factors and not by side group participation (Scheme 5).

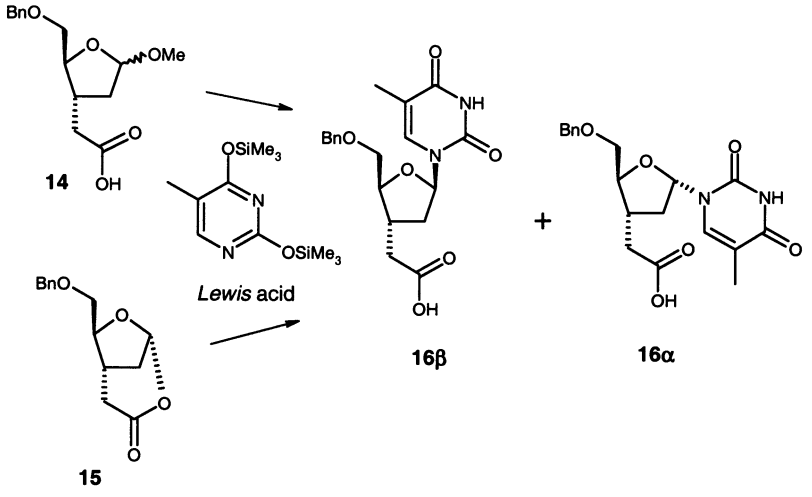
Therefore, we turned our attention to the sterically very demanding Lewis acids developed by Yamamoto.[13] A sterically very demanding Lewis acid would lead to intermediate **18** (or **17**) in which the attack from the α-face would be blocked by the bulk of the Lewis acid.



Scheme 5. Lewis acid coordination to **15**.



Scheme 6. 1 equiv. MAD, CH_2Cl_2, −78°C, then 2 equiv. $T(SiMe_3)_2$, −78→−20°C.



entry	*conditions:*	*ratio 16β : 16α :*	
	from **14**:		
1	5 eq. $SnCl_4$, CH_3CN, 65°C	5 :	2
2	5 eq. $SnCl_4$, CH_3NO_3, 0°C	2 :	1
3	5 eq. $SnTf_2$, CH_3CN, 0°C	3 :	1
4	3 eq.$YbTf_3$, CH_3CN, 0°C	2 :	1
	from **15**:		
5	no catalyst, CH_3NO_3, 95°C	3 :	2
6	1 eq. ATPH,[14] CH_2Cl_2,-20°C	45 :	1
7	1 eq. MAD,[15] CH_2Cl_2, -20°C	100 :	not detected
8	0.1 eq. MAD,[15] CH_2Cl_2, -20°C	95 :	5

Scheme 4. α/β selectivity of thymine introduction under modified Vorbrüggen conditions.

In full agreement with our analysis, the use of ATPH[13,14] as Lewis acids led to formation **16**β and **16**α in a ratio 45:1; even more impressive results were obtained with MAD,[13,15] which led to exclusive formation of the desired **16**β. Interestingly, catalytic amounts of MAD led to formation of **16** with only slightly diminished β-selectivity.

When these conditions were applied to **13**, compound **19** was obtained as a single diastereomer in 61% yield (Scheme 6).

In conclusion, we have developed a highly stereoselective synthesis of 5′-(*S*)-methyl-3′-carboxymethylene-3′-deoxythymidine. The stereochemistry at C(4′) and C(5′) has been controlled by asymmetric Sharpless bishydroxylation, while the C(3′)-stereochemistry could be efficiently controlled during cyclization of the C(3′)-pro-chiral diester **7** to the lactone **2**. Enantiomeric excess was increased to 100% through enantioselective enzymatic hydrolysis of ester **10**. In a final key step thymine introduction was accomplished with exclusive β-face selectivity through the novel use of Yamamoto's bulky aluminum based Lewis acid 'MAD' in a modified Vorbrüggen-type nucleosidation reaction. We expect that this methodology may be applied to the synthesis of related nucleoside and C-nucleoside analogs.

The use of **19** in the synthesis of novel oligonucleotide backbone modifications will be reported elsewhere.

Acknowledgements

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References

1. (a) Lebreton, J.; De Mesmaeker, A.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Tetrahedron Lett*. **1993**, *34*, 6383; (b) De Mesmaeker, A.; Waldner, A.; Lebreton, J.; Hoffmann, P.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Angew. Chem., Int. Ed. Engl*. **1994**, *33*, 226; (c) Idziak, I.; Just, G.; Damha, M. J.; Giannaris, P. A. *Tetrahedron Lett*. **1993**, *34*, 5417; (d) Wendeborn, S.; Wolf, R. M.; De Mesmaeker, A. *Tetrahedron Lett*. **1995**, *36*, 6879; (e) De Mesmaeker, A.; Haener, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res*. **1995**, *28*, 366; (f) De Mesmaeker, A.; Altmann, K.-H.; Waldner, A.; Wendeborn, S. *Curr. Opin. Struct. Biol*. **1995**, *5*, 343.
2. (a) Becker, H.; Soler, M. A.; Sharpless, K. B. *Tetrahedron* **1995**, *51*, 1345; (b) Allevi, P.; Tarocco, G.; Longo, A.; Anastasia, M.; Cajone, F. *Tetrahedron: Asymmetry* **1997**, *8*, 1315.
3. Krapcho, P. *Synthesis* **1982**, 805.
4. Mulzer, J.; Kappert, M.; Huttner, G.; Jibril, I. *Angew. Chem., Int. Ed. Engl*. **1984**, *23*, 704.
5. (a) Wessel, H.-P.; Iverson, T.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. I* **1985**, 2247; (b) Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett*. **1988**, *29*, 4139 The resulting trichloroacetamide was best removed from the crude product by Kugelrohr destillation, since it co-eluted on silica gel together with **10**.
6. Examples for selective reductions of lactols in the presence of esters can be found: (a) Binch, H.; Stangier, K.; Thiem, J. *Carbohydrate Res*. **1998**, *306*, 409; (b) Kohn, P.; Samaritano, R. H.; Lerner, L. M. *J. Am. Chem. Soc*. **1965**, *87*, 5475; (c) Tse, A.; Mansour, T. *Tetrahedron Lett*. **1995**, *36*, 7807.
7. ee's were determined by chiral HPLC; hydrolysis of the enantiomer of **10** (prepared from **4** by the same route but using β-AD-mix) with NaOH gave enantiomer of **12**, allowing unambiguous determination of enantiomeric excess by chiral HPLC.
8. See Ref. 1b.
9. Side group participation was reported to be successful in a related Vorbrüggen reaction when the thionoester was employed: Lavallée, J.-F.; Just, G. *Tetrahedron Lett*. **1991**, *32*, 3472.
10. For side group participation of methylenephosphonothioate in a related Vorbrüggen reaction see: Yokomatsu, T.; Sada, T.; Shimizu, T.; Shibuya, S. *Tetrahedron Lett*. **1998**, *39*, 6299.
11. While our studies were in progress a similar approach for the synthesis of C-glycosides from bicyclic lactones has been reported: Gaertzen, O.; Misske, A. M.; Wolbers, P.; Hoffmann, H. M. R. *Synlett* **1999**, 1041.
12. Niedballa, U.; Vorbrüggen, H. *J. Org. Chem*. **1974**, *39*, 3654.
13. (a) Maruoka, K.; Imoto, H.; Yamamoto, H. *Synlett* **1994**, *6*, 441; (b) Maruoka, K.; Imoto, H.; Saito, S.; Yamamoto, H. *J. Am. Chem. Soc*. **1994**, *116*, 4131; (c) Maruoka, K.; Shimada, I.; Imoto, H.; Yamamoto, H. *Synlett* **1994**, *7*, 519; (d) Maruako, K.; Concepcion, B.; Murase, N.; Oishi, M.; Hirayama, N.; Yamamoto, H. *J. Am. Chem. Soc*. **1993**, *115*, 3943; (e) Saito, S.; Shiozawa, M.; Yamamoto, H. *Angew. Chem., Int. Ed*. **1999**, *38*, 1769; (f) Saito, S.; Yamamoto, H. *J. Chem. Soc., Chem. Commun*. **1997**, 1585.
14. ATPH = aluminum tris(2,6-diphenylphenoxide).
15. MAD = methylaluminum bis(2,6-di-*tert*-butyl-4-methylphenoxide).