has to be protected against oxidation, this rate enhancement is remarkable. Compared to the cysteine residue in BSA ($k = 30\ min^{-1}\,M^{-1}$), the reactivity of the scRD3 cysteine residue is 670-fold higher. Two scenarios appear plausible for the mechanism of the reaction of the antibody with **3**. In the first, the two different cysteine residues react in an ordered fashion. In the second, the first reaction is followed by intramolecular disulfide exchange between the two cysteine residues before the antibody reacts with another molecule of **3**. To investigate which of the two cysteine residues Cys^{96} and Cys^{100k} in the RD3 antibody is the more reactive one and whether their reactivity is mutually dependent, each was mutated to alanine to give the mutants ^{Ala96}RD3 and ^{Ala100k}RD3. The expression level for both mutants as a Fab were significantly below that for wild-type RD3 antibody. However, 0.16 mg of the ^{Ala100k}RD3 mutant could still be isolated from one liter of shake-flask culture whereas we were not able to isolate sufficient amounts of the ^{Ala96}RD3 mutant. The ^{Ala100k}RD3 mutant possesses 0.9 equivalents of free thiol per Fab and its second-order rate constant for the reaction with **3** is $2.5 \times 10^3\ min^{-1}\,M^{-1}$. These data demonstrate that the presence of both cysteines is important for the folding of the antibody and that their reactivity is mutually dependent. The fact that the mutant ^{Ala100k}RD3 possesses a higher reactivity than the less reactive cysteine residue of wild-type RD3 antibody suggests that Cys96 is the reactive residue.

In summary, this work represents an important step in our efforts towards the generation of artificial DNA repair proteins. The established in vitro selection system and the isolated clones with reactive cysteine residues, in particular scRD3, open the way for selections of antibody-based AGTs by using derivatives of the type **1**.

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[1] A. E. Pegg, *Mutat. Res.* **2000**, *462*, 83.
[2] P. G. Schultz, R. A. Lerner, *Science* **1995**, *269*, 1835.
[3] D. Hilvert, *Annu. Rev. Biochem.* **2000**, *69*, 751.
[4] R. Damoiseaux, A. Keppler, K. Johnsson, *ChemBiochem* **2001**, *4*, 285.
[5] K. D. Janda, C. H. Lo, T. Li, C. F. Barbas, P. Wirsching, R. A. Lerner, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 2532.
[6] C. F. Barbas, W. Amberg, A. Simoncsits, T. M. Jones, R. A. Lerner, *Gene* **1993**, *137*, 57.
[7] A. Krebber, S. Bornhauser, J. Burmester, A. Honegger, J. Willuda, H. R. Bosshard, A. Plückthun, *J. Immunol. Methods* **1997**, *201*, 35.
[8] Y. Tang, N. Jiang, C. Parakh, D. Hilvert, *J. Biol. Chem.* **1996**, *271*, 15682.
[9] A. Plückthun, A. Krebber, C. Krebber, U. Horn, U. Knüpfer, R. Wenderoth, L. Nieba, K. Proba, D. Riesenberg, in *Antibody engineering* (Eds.: J. McCafferty, H. Hoogenboom, D. J. Chiswell), Oxford University Press, **1996**, pp. 203 – 249.
[10] K. M. Arndt, K. M. Muller, A. Pluckthun, *Biochemistry* **1998**, *37*, 12918.
[11] D. J. Smith, E. T. Maggio, G. L. Kenyon, *Biochemistry* **1975**, *14*, 766.

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Synthesis and NMR Structure of Peptidomimetic α4β7-Integrin Antagonists

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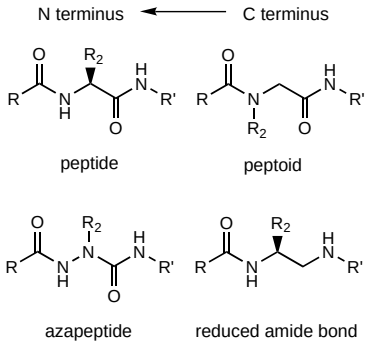
drug research · inhibitors · medicinal chemistry · peptidomimetics · structure elucidation

The development of new anti-inflammatory drugs is currently one of the great challenges in medicinal chemistry.[1] Recently, α4β1- and α4β7 integrins were shown to be promising targets for treating inflammatory and autoimmune diseases.[2–4] α4β7 integrins bind to the mucosal addressin cell adhesion molecule 1 (MAdCAM-1) through their Leu-Asp-Thr sequence (LDT sequence) specifically and with high affinity.[5, 6] An overexpression of MAdCAM-1 occurs in mouse models of colitis, in human inflammatory bowel disease (IBD), and in chronic inflammatory liver disease.[7, 8] Recently, we and others were able to derive peptidic α4β7 integrin antagonists from the natural LDT-binding sequence.[9–11] For the further development of nonpeptidic and highly selective drugs, we identified the structural and functional requirements of the LDT recognition sequence within α4β7 integrin antagonists by using several peptidomimetic variations such as peptoids, azapeptides, and reduced amide bonds (Scheme 1). An aromatic residue *N*-terminal to the LDT sequence



Scheme 1. *Comparison of different peptidomimetics.*

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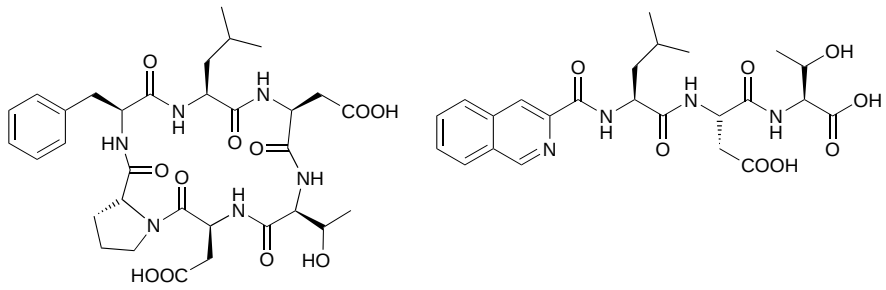

1 **10**

Scheme 2. *Recently published α4β7-integrin antagonists.*

proved to be advantageous for biological activity, therefore we also considered this position in our investigations.[10, 11] In the lead compounds **1** and **10** (Scheme 2), we systematically substituted each amino acid within the XLDT sequence (X = phenylalanine in **1**, or isoquinoline-carbonyl in **10**) by the corresponding peptoid building block (Table 1).[12, 13] These peptoid – peptide hybrids show different relative distances between side chains with respect to their position on the backbone. Furthermore, as a result of the presence of tertiary amide bonds, the hydrogen binding characteristics of the backbone are changed and the flexibility is increased.[14, 15]

The incorporation of azaamino acids in compounds **1** and **10** (Table 2) maintains the hydrogen bonding characteristics of the backbone. Theoretical studies suggest that azapeptides should be more rigid than their conventional peptide counterparts.[16–18] As a consequence, azapeptides have the potential to increase activity and selectivity. Furthermore, the incorporation of azaamino acids results in a decrease in the number of stereo centers. The configuration of an azaamino acid lies roughly between the D- and L-configuration of a normal amino acid.[19, 20]

Table 1. *Specific effects of peptidomimetics containing a peptoid building block on α4β7- and α4β1-integrin-mediated cell adhesion to MAdCAM-1 and VCAM-1.*[a]

Compound	No.	Cell adhesion [%]		
		α4β7/ MadCAM-1	α4β7/ VCAM-1	α4β1/ VCAM-1
c(F-L-D-T-D-p)	**1**	3 ± 4	38 ± 20	72 ± 10
c(Tic-L-D-T-D-p)[11]	**2**	9 ± 1	nt	nt
c(A-L-D-V-D-p)[11]	**3**	40 ± 7	nt	nt
c(Nphe-L-D-T-D-p)	**4**	19 ± 10	60 ± 28	97 ± 6
c(F-Nleu-D-T-D-p)	**5**	102 ± 5	86 ± 13	101 ± 10
c(F-L-Nasp-T-D-p)	**6**	88 ± 22	94 ± 12	nt[b]
c(F-L-D-Nval-D-p)	**7**	98 ± 20	92 ± 19	106 ± 11
H_2N-F-L-D-T-OH	**8**	98 ± 0	66 ± 8	100 ± 2
H_2N-Nphe-L-D-T-OH	**9**	87 ± 15	87 ± 16	97 ± 6
Iquin-L-D-T-OH[c]	**10**	48 ± 29	81 ± 4	nt[b]
Iquin-Nleu-D-T-OH[c]	**11**	110 ± 17	97 ± 13	95 ± 3
Iquin-L-Nasp-T-OH[c]	**12**	108 ± 4	96 ± 3	99 ± 7
Iquin-Nleu-Nasp-Nval-OH[c]	**13**	97 ± 13	80 ± 25	97 ± 5

[a] Cell adhesion is presented as a percentage of the medium control. The data represents the mean values ± standard deviation for the three experiments, each of which was repeated three times with a peptidomimetic concentration of 1 mg mL^{-1}. [b] nt = not tested. [c] Iquin = isoquinoline-4-carbonyl.

To elucidate the importance of the peptide backbone for the biological activity of α4β7-integrin antagonists, we systematically reduced the amide bonds in compounds **1** and **10** (Table 3). We also solved the NMR spectroscopy structure of the highly active cyclic peptide cyclo(Tic[1]-Leu[2]-Asp[3]-Thr[4]-Asp[5]-D-Pro[6]) (**2**) in water. In contrast to compound **1**, compound **2** contains tetrahydroisoquinoline-3-carboxylic acid (Tic), which is a constrained mimic of Phe. As the flexibility of the aromatic side chain is reduced in **2**, the orientation of the aromatic ring, which is important for the biological activity, can easily be determined.

To evaluate the biological influence of the modifications, we used a cell adhesion assay. The integrin ligands MAdCAM-1 and VCAM-1 were immobilized on tissue culture plates. The adhesion of the lymphoid cell lines 38C13-β7 (α4β7^{pos}, α4β1^{neg}) and Jurkat

Table 2. *Specific effects of peptidomimetics containing an azaamino acid on α4β7- and α4β1-integrin-mediated cell adhesion to MAdCAM-1 and VCAM-1.*[a]

Compound	No.	cell adhesion [%]		
		α4β7/ MadCAM-1	α4β7/ VCAM-1	α4β1/ VCAM-1
c(azaPhe-L-D-T-D-p)	**14**	25 ± 1	58 ± 7	89 ± 5
c(F-azaLeu-D-T-D-p)	**15**	87 ± 9	88 ± 9	100 ± 8
c(F-L-azaAsp-T-D-p)	**16**	106 ± 3	103 ± 12	99 ± 5
c(F-L-D-azaVal -D-p)	**17**	89 ± 17	nt[b]	102 ± 8
Iquin-azaPhe-L-D-T-OH[c]	**18**	130 ± 5	104 ± 8	99 ± 1
H-azaPhe-L-D-T-OH	**19**	95 ± 25	89 ± 8	101 ± 4
Iquin-azaLeu-D-T-OH[c]	**20**	92 ± 12	96 ± 2	99 ± 8
Iquin-L-azaAsp-T-OH[c]	**21**	103 ± 21	108 ± 10	102 ± 4
Iquin-L-D-azaVal-OH[c]	**22**	131 ± 27	nt[b]	nt[b]

[a] Cell adhesion is presented as a percentage of the medium control. The data represents the mean values ± standard deviation for the three experiments, each of which was repeated three times with a peptidomimetic concentration of 1 mg mL^{-1}. [b] nt = not tested. [c] Iquin = isoquinoline-4-carbonyl.

Table 3. *Specific effects of peptides containing a reduced amide bond on α4β7- and α4β1-integrin-mediated cell adhesion to MAdCAM-1 and VCAM-1.*[a]

Compound	No.	cell adhesion [%]		
		α4β7/ MadCAM-1	α4β7/ VCAM-1	α4β1/ VCAM-1
c(FΨ(CH_2NH)-L-D-T-D-p)[b]	**23**	113 ± 19	102 ± 3	98 ± 9
c(F-LΨ(CH_2NH)-D-T-D-p)[b]	**24**	106 ± 7	100 ± 5	98 ± 0
c(F-L-DΨ(CH_2NH)-T-D-p)[b]	**25**	114 ± 13	108 ± 2	99 ± 7
c(F-L-D-TΨ(CH_2NH)-D-p)[b]	**26**	36 ± 19	95 ± 5	78 ± 19
1-NaphthylΨ(CH_2NH)-L-D-T-OH[b]	**27**	86 ± 15	97 ± 8	102 ± 6
2-NaphthylΨ(CH_2NH)-L-D-T-OH[b]	**28**	122 ± 26	103 ± 2	103 ± 1
Iquin-LΨ(CH_2NH)-D-T-OH[b,c]	**29**	102 ± 23	97 ± 4	98 ± 7
Iquin-L-DΨ(CH_2NH)-T-OH[b,c]	**30**	81 ± 4	106 ± 2	97 ± 4

[a] Cell adhesion is presented as a percentage of the medium control. The data represents the mean values ± standard deviation for the three experiments, each of which was repeated three times with a peptidomimetic concentration of 1 mg mL^{-1}. [b] Ψ indicates the presence of a reduced amide bond. [c] Iquin = isoquinoline-4-carbonyl.

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($\alpha4\beta1^{pos}$, $\alpha4\beta7^{neg}$) were analyzed in the presence or absence of the peptidomimetics at a final concentration of 1 mg mL^{-1}. The results are summarized in Tables 1, 2, and 3. The inhibitory activities of the well-known $\alpha4\beta7$ integrin antagonists **1** and **10** were measured as a positive control. None of the tested compounds inhibited the adhesion of $\alpha4\beta1$-integrin-expressing Jurkat cells to vascular cell adhesion molecule 1 (VCAM-1). This test system thus showed that, if active, the tested compounds were highly selective inhibitors for $\alpha4\beta7$ integrin. In addition, these results exclude potential toxic effects of the compounds.

The biological evaluation of the peptidomimetics revealed that some cyclic compounds (**4**, **14**, and **26**) maintained their biological activity and selectivity towards $\alpha4\beta7$ integrins, especially when modifications were *N*-terminal to the LDT recognition sequence (Figure 1). The Phe^1 residue in cyclo-(Phe-

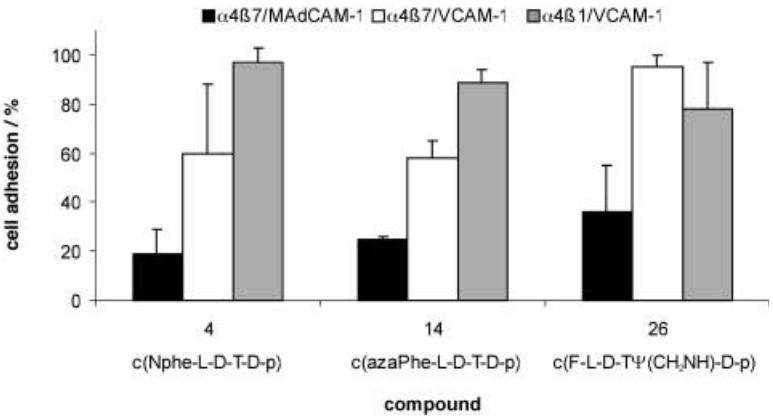

Figure 1. *Adhesion of 38C13-$\beta7$ lymphoid cell line ($\alpha4\beta7$) and Jurkat cells ($\alpha4\beta1$) to microtiter plates coated with MAdCAM-1 and VCAM-1 after incubation with antagonists. The results are shown as the mean $\pm$ standard deviation.*

Leu-Asp-Thr-Asp-D-Pro) (**1**) could be substituted by the corresponding peptoid building block Nphe (**4**) and the corresponding azaamino acid azaPhe (**14**) and activity was retained. In contrast, all *N*-terminal modifications in the linear compound (**10**) resulted in completely inactive compounds (**8**, **9**, **18**, **19**, **27**, **28**). The linear peptoid isoquinoline-3-carbonyl-Nleu-Nasp-Nval-OH (**13**) also showed no activity. This supports the assumption that not only the correct distances between the functional groups are essential for biological activity of $\alpha4\beta7$ integrin antagonists, but that the peptide backbone may also influence binding to the receptor. This hypothesis is also supported by the fact that neither the linear compounds with a reduced amide bond nor cyclo-(PheΨ(CH$_2$NH)-Leu-Asp-Thr-Asp-D-Pro) (**23**) showed any biological activity. Only the amide bond between Thr^4 and Asp^5 in cyclo-(Phe-Leu-Asp-ThrΨ(CH$_2$NH)-Asp-D-Pro) (**26**) seemed to be nonessential. It is conceivable that the importance of the other amide bonds in the cyclic peptides is not only a result of interactions with the integrin, but also, or even exclusively, that they are needed to stabilize the cyclic structure through hydrogen bonds. The biological activity of compound **26** also shows that besides of *N*-terminal modifications, some variations of the Thr^4 residue within the LDT recognition sequence are also possible. In our previous work we were already able to substitute the Thr^4 residue in compound **1** by Val to give compound **3** without loss of activity.[11] Compounds **4**, **14**, and **26** are selective for $\alpha4\beta7$ integrins, with a preference for inhibition of the $\alpha4\beta7$-integrin – MAdCAM-1 interaction. The IC_{50} values of the biologically active compounds are shown in Table 4.

Table 4. *IC_{50} values of $\alpha4\beta7$-integrin antagonists.*

Compound	No.	IC_{50} [μM]
c(azaPhe-L-D-T-D-p)	**14**	61 ± 21
c(F-L-D-TΨ(CH$_2$NH)D-p)	**26**	137 ± 48

In some cases the linear peptidomimetic is inactive, while the corresponding cyclic peptidiomimetic is still active, as indicated in Tables 1, 2, and 3. It seems reasonable to assume that the LDT conformation in the ring is very close to the bioactive conformation. For structure elucidation, we used the highly active, constrained cyclic hexapeptide cyclo-(Tic-Leu-Asp-Thr-Asp-D-Pro) **2**. NMR spectroscopy data were obtained in water at 300 K. Distance geometry (DG) calculations[21, 22] and molecular dynamics (MD) simulations[23] showed that D-Pro^6 occupies position i + 1 in a βII′-turn. ROE spectroscopy data suggested a flip of the peptide bond between Asp^3 and Thr^4, which indicates that there might be at least two conformations. The flip of the peptide bond between Asp^3 and Thr^4 could clearly be observed after approximately 130 picoseconds of simulation time with a time-averaged distance restraints protocol[24–26]. The two conformations were averaged and subsequently minimized to give the structures depicted in Figure 2. The position of the aromatic ring of Tic^1, which is very important for the biological activity, is nearly perpendicular to the peptide ring.

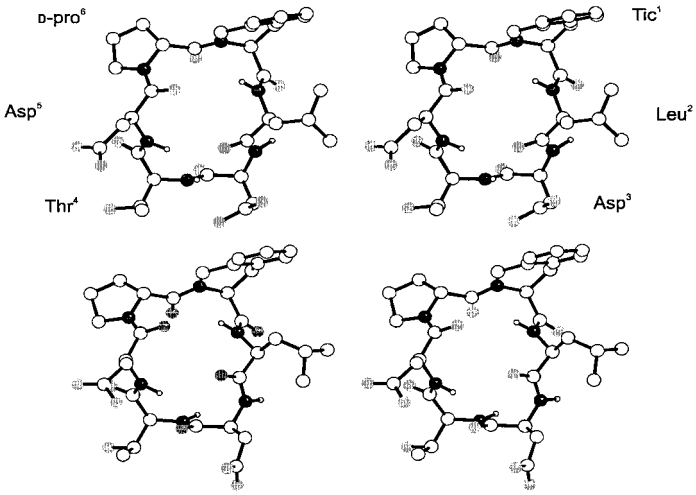

Figure 2. *Stereoplot of the solution structures of the two conformers of cyclo-(Tic-Leu-Asp-Thr-Asp-D-Pro) (**2**). The structures were determined by a 300 ps dynamics simulation with time-averaged distance restraints. D-Pro^6 occupies position i + 1 in a βII′ turn. The spatial position of Tic^1 is the same in both conformers, whereas the peptide bond between Asp^3 and Thr^4 is flipped.*

In summary, these results show that within the LDT recognition sequence in α4β7 integrin antagonists the position of Leu and Asp are invariant for common peptide modifications. Some variations are possible in both the N- and C-terminal directions from this core unit. NMR spectroscopy data indicate that the C-terminal aromatic system, which is important for the biological activity, should be perpendicular to the peptide backbone. The biological data provide important information for the design of novel and highly active α4β7 integrin antagonists. Further efforts to reduce the peptidic character of these peptidomimetics could lead to drug candidates for the treatment of inflammatory diseases.

[1] A. J. Lewis, A. M. Manning, *Curr. Opin. Chem. Biol.* **1999**, *3,* 489 – 494.
[2] R. R. Lobb, S. P. Adams, *Expert Opin. Invest. Drugs* **1999**, *8,* 935 – 945.
[3] P. Hesterberg, D. Winsor-Hines, M. J. Briskin, D. Soler-Ferran, C. Merrill, C. R. Mackay, W. Newman, D. J. Ringler, *Gastroenterology* **1996**, *111,* 1373 – 1380.
[4] S. Kato, R. Hokari, K. Matsuzaki, A. Iwai, A. Kawaguchi, S. Nagao, T. Miyahara, K. Itoh, H. Ishii, S. Miura, *J. Pharmacol. Exp. Ther.* **2000**, *295*, 183 – 189.
[5] M. J. Briskin, L. Rott, E. C. Butcher, *J. Immunol.* **1996**, *156,* 719 – 726.
[6] J. L. Viney, S. Joenes, H. H. Chiu, B. Lagrimas, M. E. Renz, L. G. Presta, D. Jackson, K. J. Hillan, S. Lew, S. Fong, *J. Immunol.* **1996**, *157,* 2488 – 2497.
[7] A. J. Grant, P. F. Lalor, S. G. Hübscher, M. Briskin, D. H. Adams, *Hepatology* **2001**, *33*, 1065 – 1072.
[8] M. Briskin, D. Winsor-Hines, A. Shyjan, N. Cochran, S. Bloom, J. Wilson, L. M. McEvoy, E. C. Butcher, N. Kassam, C. R. Mackay, W. Newman, D. J. Ringler, *Am. J. Pathol.* **1997**, *151*, 97 – 110.
[9] H. N. Shroff, C. F. Schwender, D. Dottavio, L. L. Yang, M. J. Briskin, *Bioorg. Med. Chem. Lett.* **1996**, 2495 – 2500.
[10] H. N. Shroff, C. F. Schwender, A. D. Baxter, F. Brookfield, L. J. Payne, N. A. Cochran, D. L. Gallant, M. J. Briskin, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1601 – 1606.
[11] J. Boer, D. Gottschling, A. Schuster, M. Semmrich, B. Holzmann, H. Kessler, *J. Med. Chem.* **2001**, *44*, 2586 – 2592.
[12] R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Wang, S. Rosenberg, C. K. Marlowe, D. C. Spellmeyer, R. Tan, A. D. Frankel, D. V. Santi, F. E. Cohen, P. A. Bartlett, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 9367 – 9371.
[13] H. Kessler, *Angew. Chem.* **1993**, *105*, 572 – 573; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 543 – 544.
[14] P. Armand, K. Kirshenbaum, R. A. Goldsmith, S. Farr-Jones, A. E. Barron, K. T. V. Truong, K. A. Dill, D. F. Mierke, F. E. Cohen, R. N. Zuckermann, E. K. Bradley, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 4309 – 4314.
[15] E. K. Bradley, *J. Magn. Reson., Ser. B* **1996**, *110*, 195 – 197.
[16] J. Gante, *Synthesis* **1989**, 405 – 413.
[17] C. H. Reynolds, R. E. Hormann, *J. Am. Chem. Soc.* **1996**, *118*, 9395 – 9401.
[18] H.-J. Lee, I.-A. Ahn, S. Ro, K.-H. Choi, Y.-S. Choi, K.-B. Lee, *J. Pept. Res.* **2000**, *56*, 35 – 46.
[19] F. André, A. Vicherat, G. Boussard, A. Aubry, M. Marraud, *J. Pept. Res.* **1997**, *50*, 372 – 381.
[20] F. André, G. Boussard, D. Bayeul, C. Didierjean, C. Aubry, M. Marraud, *J. Pept. Res.* **1997**, *49*, 556 – 562.
[21] DISGEO, Quantum Chemistry Exchange Program, Exchange No. 507, Indiana University, **1986**.
[22] T. F. Havel, *Distance Geometry and Molecular Conformation*, John Wiley & Sons, New York, **1988**.
[23] A. T. Hagler in *Molecular Dynamics and Protein Structure* (Ed.: J. Hermans), Polycrystal Book Service, Western Springs, **1985**, pp. 133 – 138.
[24] A. E. Torda, R. M. Scheek, W. F. v. Gunsteren, *Chem. Phys. Lett.* **1989**, *157,* 289 – 294.
[25] A. P. Nanzer, W. F. v. Gunsteren, A. E. Torda , *J. Biomol. NMR* **1995**, *6*, 313 – 320.
[26] C. Roelz, PhD thesis, Technische Universität München, Germany, **2000**.

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Varied DNA Polymerase—Substrate Interactions in the Nucleotide Binding Pocket

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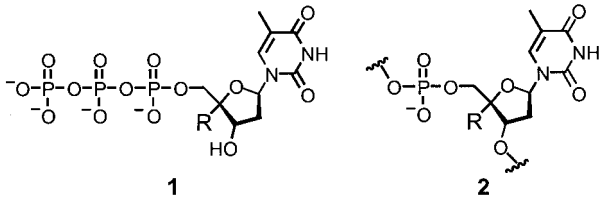
DNA polymerases · DNA recognition · DNA replication · nucleotide analogues · nucleotides

Faithful transfer of genetic information from one generation to its offspring is crucial for the survival of any living species. Genomic integrity relies greatly upon the ability of DNA polymerases to efficiently catalyze selective DNA synthesis in a template-directed manner.[1] Despite enormous efforts in structural and functional studies, the complex mechanisms by which DNA polymerases ensure selective DNA synthesis are not fully understood.[2, 3] Recently, we introduced a functional strategy to monitor enzyme interactions that act on the sugar moiety of an incoming triphosphate within the nucleotide binding pocket of the Klenow fragment (Kf) of *Escherischia coli* DNA polymerase I (exo$^-$ mutant).[4] We found that the nucleotide insertion selectivity of Kf$^-$ is increased approximately hundredfold when modified sugars **1b**, **c** (Scheme 1) are employed. This result strongly suggests the involvement of steric constraints, which act on the 2′-deoxyribose of an incoming nucleoside triphosphate in Kf$^-$ selectivity processes.



Scheme 1. *Thymidine analogues used as steric probes in functional DNA polymerase studies.*

Further, new valuable insights into selective Kf$^-$ interactions with the sugar backbone in the minor groove were obtained through site-specific incorporation of thymidines **2a**–**d** into primer template substrates.[5] Herein, we investigate the interplay of Kf$^-$ with the 2′-deoxyribose moiety of the coding nucleotide by using steric probes **2a**–**d** in functional enzyme investigations. This is a particularly intriguing topic since

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