Pergamon

Tetrahedron Letters 42 (2001) 6511–6513

TETRAHEDRON LETTERS

Synthesis of a dexamethasone-21-maleimido-linked derivative as a potential molecule for specific gene delivery†

A. Bernasconi,[a,‡] A. Rebuffat,[a,‡] P. Bigler,[b] F. J. Frey[a] and B. M. Frey[a,*]

[a]*Division of Nephrology and Hypertension, Department of Clinical Research, University of Berne, Inselspital, Berne, Switzerland*
[b]*Department of Chemistry and Biochemistry, University of Berne, 3010 Berne, Switzerland*

Received 7 June 2001; accepted 12 July 2001

Abstract—The synthesis of the dexamethasone-21-maleimido-linked derivative **5** is described for the first time. The two principal steps of this synthesis are (1) the formation of a stable urethane **3** and (2) the introduction of a reactive maleimido group via a linker to get **5**. This novel compound **5** is designed to examine the interaction of the steroid with other relevant molecules or functional groups, via the formation of conjugates. The structure of **5** was proven by NMR, taking advantage of a newly developed method (HMSC). © 2001 Elsevier Science Ltd. All rights reserved.

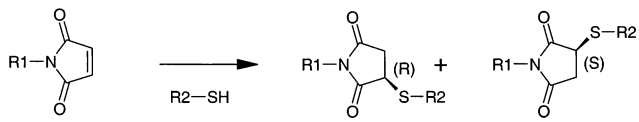
The synthetic steroid dexamethasone (Dex) (**1**, Scheme 2), is a member of the steroid family. It demonstrates a strong binding affinity for the glucocorticoid receptor (GR) and is able to induce the translocation of GR from the cytoplasm to the nucleus.[1–3] Therefore, it has been proposed that Dex might be a potential molecule to improve delivery of *endo*- and xenobiotics. This strategy explores the possibility to exploit nuclear receptors as carriers for drugs and DNA transport. The variability of steroid molecules and the cell specificity makes this approach interesting for pharmacotherapy and gene therapy of disease states with a distinct pattern of steroid receptor expression such as cancer. For this purpose a stable modification of the steroid molecule is needed.

We describe in this paper the synthesis of a steroid derivative **5** which is able to interact with sulfhydryl groups of either modified oligonucleotides or proteins[4–6] or peptide nucleic acids (PNAs) (Scheme 1).[7]

The classical conjugate addition of thiol groups on maleimide (**A**) may result in two biologically interesting diastereoisomers (**B** and **C**), as in the case of **5**, which are stable under physiological conditions and may be used for cell culture assay.

The synthesis of the 21-maleimido-linked steroid is based on the reactive commercially available compound, *N*-(γ-maleimidobutyryloxy)succinimide (GMBS-Pierce). On one hand this bifunctional molecule reacts with the amino-steroid **4** and on the other hand it allows the activation of the so formed steroid-derivative **5** for further reactions of the maleimido group with thiols.

At the beginning the hydroxyl group at position 21 of dexamethasone **1** (Scheme 2) is activated by 4-nitrophenylchloroformate, in the presence of *N*-methylmorpholine (NMM) in tetrahydrofuran (THF), to afford **2** in 55% yield. The resulting active ester **2** is treated with mono-*N*-Boc-1,6-diaminohexanhydrochlorid (commercially available), in DMF/NMM overnight at room temperature, leading to compound **3** (97% yield).



A B C

Enantiomers

(diastereoisomers in the case of **5**)

Scheme 1. The reaction scheme of maleimide with thiol groups.

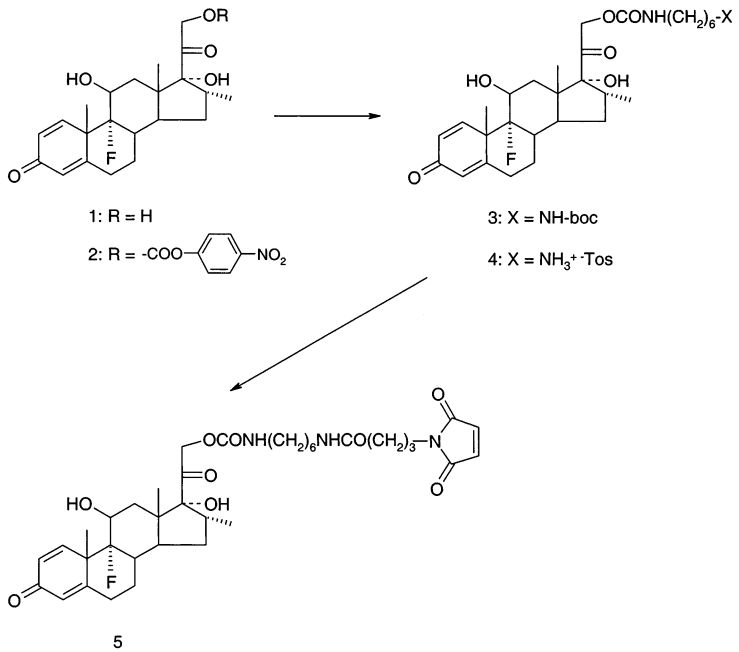
Keywords: dexamethasone; glucocorticoid receptor; urethane bond; maleimide.

* Corresponding author. E-mail: brigitte.frey@dkf2.unibe.ch

† This work was supported by a grant from the Swiss National Foundation for Scientific Research (no. 4037-44802).

‡ Both authors contributed equally to the work.

PII: S0040-4039(01)01291-6



Scheme 2. *Reagents and conditions*: (a) 3 equiv. 4-nitrophenyl chloroformate, 3.5 equiv. NMM, THF, 24 h, rt, 55%; (b) 1.5 equiv. mono-*N*-Boc-1,6-diaminohexanhydrochlorid, 2 equiv. NMM, DMF, 24 h, rt, 97%; (c) 2.5 equiv. toluene-4-sulfonic acid monohydrate, acetonitrile, 24 h, rt, 71%; (d) 1.5 equiv. *N*-(γ-maleimidobutyryloxy)succinimide, 2 equiv. *N*-ethyldiisopropylamine, DMF, 2 h, rt, 77%.

The Boc group of **3** was then removed with 4-toluene sulfonic acid in acetonitrile. The resulting ammonium salt **4** was converted to the final product **5** (77% yield) by reaction with succinimidyl 4-maleimidobutyrate in DMF and in the presence of *N*-ethyldiisopropylamine.

Spectroscopic characterization of product **5** was achieved via several standard NMR spectra (^{1}H, ^{13}C, ^{13}C DEPT and ^{1}H–^{1}H COSY). Most valuable, however, especially for unambiguously proving the connectivities at the central urethane and the terminal maleimido group, was the application of heteronuclear two-dimensional experiments. A newly proposed method, HMSC (heteronuclear multiple bond and single bond correlation)[8] has been applied. This method simultaneously detects one-bond ($^1J_{CH}$) and long-range ($^nJ_{CH}$) proton–carbon coupling interactions within one experiment and allows the two types of coupling interactions to be separated most effectively. The corresponding spectra were calculated by simple data processing. Fig. 1 shows expansions of these two spectra with the ^{1}H and ^{13}C signals in the region of the urethane group (H-21–H25 and C20–C-25, respectively). In the $^nJ_{CH}$ connectivity spectrum (Fig. 1, top) C-23 shows $^3J_{CH}$ cross peaks with protons H-21 and H-25 and allows the two substituents at the urethane bond to be connected unambiguously. The corresponding carbon signals of these protons (C-21 and C-25) may easily be assigned taking advantage of the $^1J_{CH}$ connectivity spectrum (Fig. 1, bottom).

Experiments to convert the 21-maleimido-steroid (**5**) to biologically interesting thioether-derivatives are under investigation.

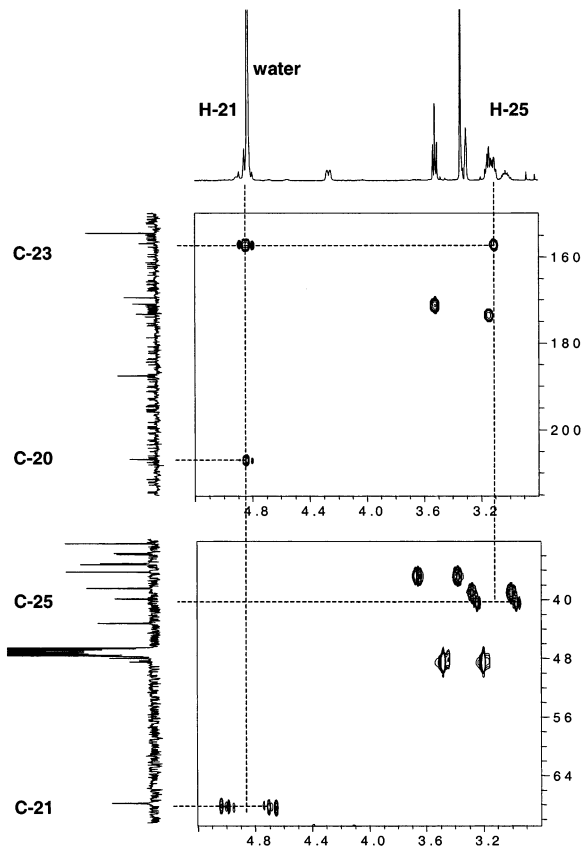


Figure 1. Expansions of the two subspectra of a HMSC experiment showing $^nJ_{CH}$-(top) and $^1J_{CH}$-coupling interactions (bottom) between protons and carbons of compound (**5**) dissolved in methanol-d_4.

Acknowledgements

We thank Dr. H. U. Wehrli for helpful discussions and Dr. C Mueller for performing NMR analysis. This research was supported by the Swiss National Foundation for Scientific Research No.4037-44802.

References

1. Schmidt, T. J.; Litwack, G. *Physiol. Rev.* **1982**, *62*, 1131–1192.
2. Litwack, G. *Cancer Res.* **1988**, *48*, 2636–2640.
3. Bodine, P. V.; Litwack, G. *J. Biol. Chem.* **1990**, *265*, 9544–9554.
4. Smyth, D. G.; Blumenfeld, O. O.; Konigsberg, W. *Biochem. J.* **1964**, *91*, 589–595.
5. Yoshitake, S.; Yamada, Y.; Ishikawa, E.; Masseyeff, R. *Eur. J. Biochem.* **1979**, *101*, 395–399.
6. Hashida, S.; Imagawa, M.; Inoue, S.; Ruan, K. H.; Ishikawa, E. *J. Appl. Biochem.* **1984**, *6*, 56–63.
7. Rebuffat, A.; Bernasconi, A.; Nielsen, P. E.; Bernal-Mendez, E.; Nawrocki, A.; Frey, B. M.; Frey, F. J., manuscript in preparation.
8. Burger, R.; Schorn, C.; Bigler, P. *J. Magn. Reson.* **2001**, *148*, 88–94.