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Semisynthesis of RPR 121056A, a Major Metabolite of Irinotecan (CPT-11)

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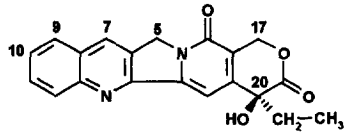
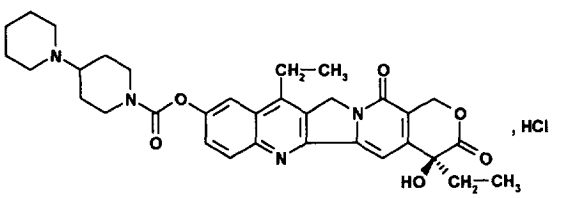
Abstract: The semisynthesis of RPR 121056A (**4**), a major metabolite of irinotecan (CPT-11, **2**), is reported starting from SN-38 (**3**) and an appropriate side-chain precusor, and using a 2-step sequence. This semisynthesis is based on the 10-O-acylation of SN-38 with the conveniently protected carbamoylchloride derivative **10** followed by cleavage of the benzylic protecting groups by hydrogenolysis. Preliminary *in vitro* results show that RPR 121056A displays no cytotoxicity.

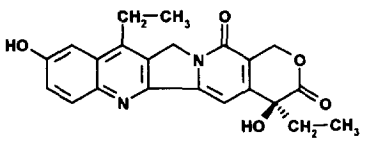
Camptothecine **1**, originally isolated by Wall et al. from *Camptotheca acuminata*[1], was the first member of a new class of antitumor agents which continue to be the subject of intensive interest because of their ability to selectively inhibit DNA topoisomerase I[2] and because of their clinical activity against solid tumors. Among the most successful examples of this class of compounds is the 7,10-disubstituted analog irinotecan (CPT-11, **2**)[3], a semisynthetic and water soluble derivative of camptothecine.



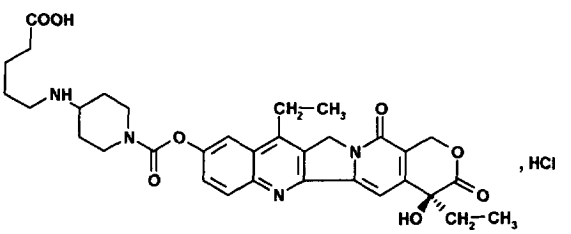
Camptothecine **1**



Irinotecan (CPT-11, **2**)



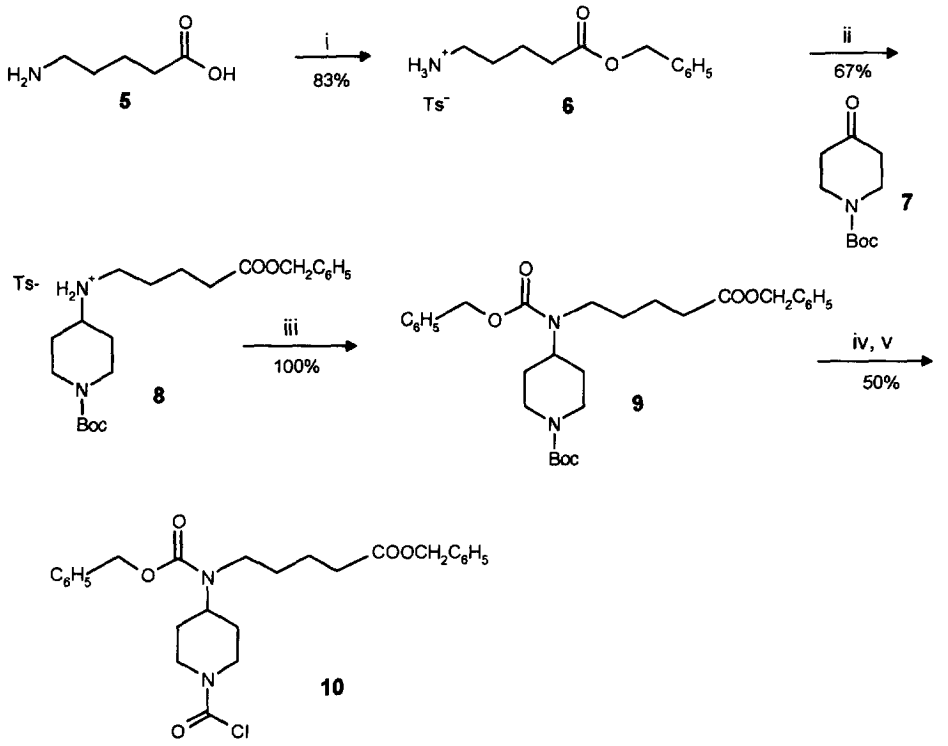
SN-38 **3**



RPR 121056A **4**

Irinotecan has been already marketed in early 1994 in Japan for the treatment of a broad range of tumors including non-small cell lung cancer, ovarian, breast, cervix and colorectal cancers as well as lymphoma. Irinotecan has been recently approved in France for the treatment of refractory colon cancer.

Scheme 1

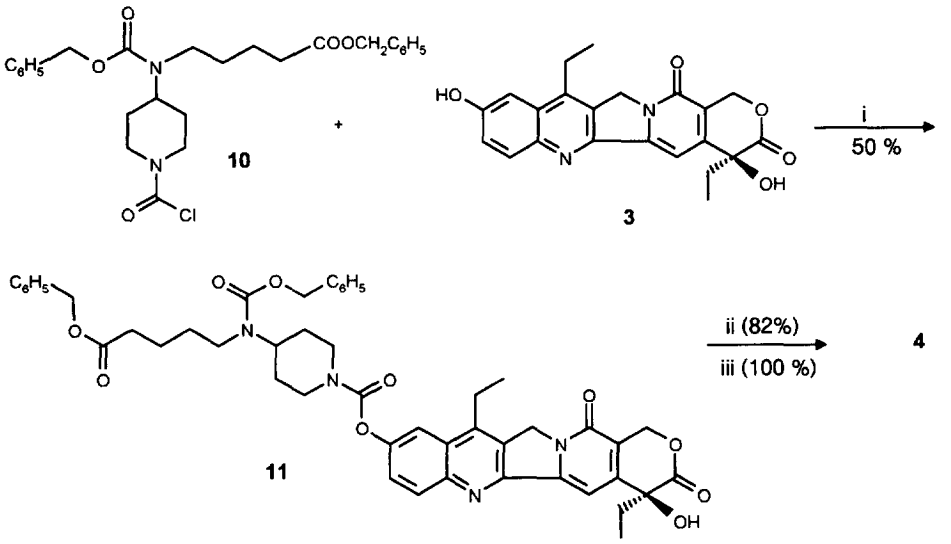


Reagents: i) benzyl alcohol, PTSA, TsCl, 80°C, 2.5h. ii) CH_2Cl_2, AcOH, $NaBH(OAc)_3$, 25°C, 3h. iii) AcOEt, $NaHCO_3$, H_2O then $ClCOOCH_2C_6H_5$, 25°C, 4h. iv) HCOOH, 25°C, 1h. v) CH_2Cl_2, Et_3N, $ClCOOCCl_3$, -10°C, 2.5h.

Irinotecan, which displays very poor *in vitro* activity, is considered to act as a prodrug and is thought to exert its antitumor properties through an *in vivo* bioactivation to give the very potent DNA topoisomerase I inhibitor SN-38 (7-ethyl-10-hydroxycamptothecine, **3**)[4]. Several groups have been involved in the identification of human metabolites in patients treated with irinotecan. Recently Robert's group first described the presence of high concentrations of a β-glucuronide of SN-38 in plasma of treated patients along with an unknown major metabolite[5]. Conjugated efforts led us to report its structure using 600 MHz NMR experiments[6]. This metabolite is very likely the result of an initial hydroxylation α to the nitrogen atom of the distal piperidine of ironotecan followed by a further oxidation leading to the opening of the intermediate hydroxypiperidine to give the corresponding δ-amino-acid **4**. Given the high interest in

elucidating the metabolic pathways of irinotecan and determining the biological and toxicological profile of the major metabolite, we undertook its preparation on a large scale basis.

Scheme 2



Reagents: i) C_5H_5N, 20°C, 20h. ii) H_2, $Pd(OH)_2$, AcOH, MeOH, 20°C, 15 psi. iii) toluene - azeotropic distillation then HCl (0.1N, 1.2 equiv.), lyophilization.

We report herein the semisynthetic scheme used for the preparation of **4**, named RPR 121056A, in order to confirm its structure and permit a complete biological evaluation. RPR 121056A was synthesized starting from SN-38 which is easily accessible in 3 steps from camptothecine[3]. The side chain attachment for the 10-position of SN-38 was prepared, as the acylating precursor **10**, using a 5-step sequence (scheme 1). Thus 5-aminopentanoic acid **5** was treated with benzyl alcohol (as the solvent) in the presence of tosylchloride and *para*-toluenesulfonic acid similarly to Arai's procedure[7] to give, after filtration from the reaction mixture, aminoester salt **6**. Because of the tendency of **6** to cyclize into the 6-membered ring lactam under neutral or basic conditions, subsequent coupling with the piperidine moiety was realized using a reductive amination of N-Boc-4-piperidinone **7**[8,9] in acidic medium, thus leading to 4-amino-piperidine derivative **8** in satisfactory yield. Compound **8** was then converted to the fully protected side-chain precursor **9** which, after cleavage of the Boc and reaction with diphosgene, afforded carbamoylchoride derivative **10**.

The coupling of **10** with SN-38 was performed in pyridine following Sawada's procedure[3] (scheme 2). The final cleavage of the benzyl and benzyloxycarbonyl protecting groups was achieved by hydrogenolysis to give the expected reduction product as a solvate with acetic acid. Hydrochloride **4** was obtained after elimination of the remaining acetic acid in refluxing toluene followed by addition of one equivalent of aqueous hydrochloric acid and freeze-drying[10].

Semisynthetic **4** was analytically identical to the metabolite isolated from human plasma of treated patients. A preliminary biological evaluation showed that **4**, like irinotecan, is devoid of cytotoxicity against tumor cells *in vitro* and displays no DNA-topoisomerase I inhibition in the *in vitro* cleavable complex assay. The evaluation of the compound's *in vivo* biological profile is in progress.

Acknowledgements: We thank Drs. S. Sable and B. Monegier du Sorbier for fruitful discussions about the structural determination of compound **4** and Drs. J.F. Riou and F. Lavelle for preliminary *in vitro* biological results. We also gratefully acknowledge E. Bouley for the technical assistance and Dr. C. Burns for critical reading of the manuscript.

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10. Yields are not optimized. All new compounds exhibit IR, ^{1}H-NMR spectra and mass spectra in agreement with the structure indicated. We report herein the ^{1}H-NMR data of metabolite **4**.
4: yellow powder, ^{1}H-NMR (400 MHz, DMSO-d_6); δ in ppm: 0,90 (t, J = 7,5 Hz, 3H : -$CH_2C\underline{H}_3$ at C-20); 1,32 (t, J = 7,5 Hz, 3H : -$CH_2C\underline{H}_3$ at C-7); 1,45-1,65 and 2.11 (2 mts, 6H and 2H respectively: -NH-$CH_2C\underline{H}_2C\underline{H}_2CH_2$-COOH and 2 -$C\underline{H}_2$- of the piperidine ring) ; 1,90 (mt, 2H : -$C\underline{H}_2CH_3$ at C-20); 2,30 (t, J = 7 Hz, 2H : -NH-$CH_2CH_2CH_2C\underline{H}_2$-COOH); 2,93 (mt : 2H : -NH-$C\underline{H}_2CH_2CH_2CH_2$-COOH); 2,90-3,30 (mts : 5H : axial-OCON-$C\underline{H}_2$- of the piperidine ring, -C$\underline{H}$- of the piperidine ring and -$C\underline{H}_2CH_3$ at C-7); 4.15 and 4.33 (2 broad d : J = 12 Hz , 1H each: equatorial-OCON-$C\underline{H}_2$- of the piperidine ring); 5,37 and 5,45 (2 s, 2H each : -$NC\underline{H}_2$- at C-5 et -$C\underline{H}_2O$- at C-17); 7,35 (s, 1H : -$\underline{H}$ at C-14); 7,70 (dd, J = 8,5 and 2 Hz, 1H : -$\underline{H}$ at C-11); 8,02 (d, J = 2 Hz, 1H : -$\underline{H}$ at C-9); 8,22 (d, J = 8,5 Hz, 1H : -$\underline{H}$ at C-12).

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