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Optimization of a 1,5-dihydrobenzo[*b*][1,4]diazepine-2,4-dione series of HIV capsid assembly inhibitors 1: Addressing configurational instability through scaffold modification

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ABSTRACT

The optimization of a 1,5-dihydrobenzo[*b*][1,4]diazepine-2,4-dione series of inhibitors of HIV-1 capsid assembly that possess a labile stereocenter at C3 is described. Quaternization of the C3 position of compound **1** in order to prevent racemization gave compound **2**, which was inactive in our capsid disassembly assay. A likely explanation for this finding was revealed by in silico analysis predicting a dramatic increase in energy of the bioactive conformation upon quaternization of the C3 position. Replacement of the C3 of the diazepine ring with a nitrogen atom to give the 1,5-dihydro-benzo[*f*][1,3,5]triazepine-2,4-dione analog **4** was well tolerated. Introduction of a rigid spirocyclic system at the C3 position gave configurationally stable 1,5-dihydrobenzo[*b*][1,4]diazepine-2,4-dione analog **5**, which was able to access the bioactive conformation without a severe energetic penalty and inhibit capsid assembly. Preliminary structure–activity relationships (SAR) and X-ray crystallographic data show that knowledge from the 1,5-dihydrobenzo[*b*][1,4]diazepine-2,4-dione series of inhibitors of HIV-1 capsid assembly can be transferred to these new scaffolds.

After nearly three decades of an intense search for new and better options for prevention and treatment of acquired immune deficiency syndrome (AIDS) and its underlying causative agent, the human immunodeficiency virus, significant progress is still required if modern medicine is to truly bring this pandemic under control.[1,2] Recent effort has centered on the search for new antiretroviral agents that operate through novel mechanisms of action in order to provide drugs with unique resistance profiles.[3] Significant progress has been made in this area, as demonstrated by the approval of the CCR5 antagonist maraviroc[4] and inhibitors of the viral enzyme integrase raltegravir and elvitegravir.[5] Recently, we reported on the discovery of a novel class of HIV replication inhibitors exemplified by compound **1** that operate through a novel mechanism of action.[6] This series of compounds bind to the N-terminal domain (NTD) of the viral capsid protein (CA), which prevents its assembly into the cone-shaped capsid core required for virion maturation and viral infectivity.[7] Herein we outline efforts to optimize lead compound **1**.

Our lead structure is characterized by a benzodiazepine core substituted at positions 3 and 5 with phenyl rings. Our earlier account revealed that only the phenyl substituent at C3 was tolerated. At the outset of optimization of compound **1**, we became concerned that, under physiological conditions, the configuration of the chiral center created by substitution at C3 was unstable. Indeed, deprotonation of a close analog of compound **1** was easily observed at elevated pH by ^{1}H NMR (data not shown). In order to prevent potential problems associated with a configurationally unstable chiral center, we prepared the quaternized analog **2** and measured its ability to inhibit capsid assembly.[8,9] Table 1 compares the profile of compounds **1** and **2** and illustrates that methylation of C3 eliminates activity against capsid assembly. Seeking an explanation for this result, we subjected compounds **1** and **2** to molecular modeling in order to compare their predicted free-state conformations. The inhibitors were first minimized with molecular mechanics[10] followed by quantum mechanics (QM) at two levels of theory; 1. DFT at the B3LYP/6-31G** level, then 2. LMP2 with a 6-31+G basis set.[11] Figure 1 shows that, at both levels of theory, compound **1** should exist as a mixture of the pseudo-equatorial and pseudo-axial conformation in varying ratios depending on

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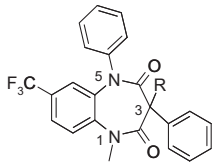
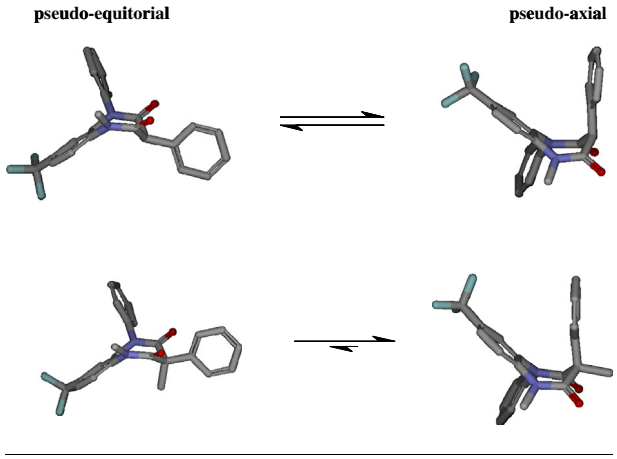
Table 1
Impact of quaternization at C3



Compound	R	IC_{50} (μM)
1	H	0.68
2	Me	8% Inhibition @ 3.5



	R	$\Delta E_{axial\text{-}equatorial}$ [kcal/mol]			
		DFT		LMP2	
		Gas phase	Solution	Gas phase	Solution
1	H	2.38	2.94	-0.06	1.31
2	Me	-8.69	-7.5	-11.44	-9.00

Figure 1. Conformational analysis of compounds **1** and **2**.

the level of theory used. Conversely, the pseudo-axial conformation of compound **2** is dramatically favored, as predicted at both levels of theory.

Consistent with these results, we have previously reported that compound **1** was detected as a mixture of magnetically non-equivalent conformations by ^{1}H NMR. Conversely, only a single set of resonances was observed for compound **2**, consistent with the >1,000,000:1 ratio predicted by quantum mechanics calculations. As previously reported, the bioactive conformation of the 3-phenylbenzodiazepine class of capsid assembly inhibitors is pseudo-equatorial.[8] Examination of the four discreet conformations suggests that the difference in ΔE observed between the two cases might be explained by a dominant steric clash ($A^{1,3}$-strain) between the C3-phenyl ring and the C3-methyl group of compound **2**, which would favor the pseudo-axial conformation. In the absence of this strain as in compound **1**, an equilibrium population of both conformations is achievable and the compound can bind to CA_{NTD}. Therefore, we conclude that the difference in potency between compounds **1** and **2** can be explained by the inability of compound **2** to adopt the bioactive conformation required to bind to CA_{NTD} and that quaternization of C3 is not a viable solution for stabilizing the configuration of this stereocenter.

At this stage, we envisaged two possibilities for modification of the C3 stereocenter. The first was to replace C3 with a nitrogen atom to give benzotriazepine **4**, a compound devoid of a chiral center and its associated problems (Fig. 2). The second was based on the recognition that, in order to disfavor the preferred conformation of a quaternized analog similar to compound **2**, we would need to maintain a torsion angle of the C3–Ph bond similar to that in the pseudoequatorial conformations, but remove the $A^{1,3}$-strain highlighted for compound **2**. Thus a strategy to achieve the correct conformation of the phenyl ring in the presence of a second substituent might be to form a spirocyclic ring system such as that shown in compound **5**. This approach was suggested by molecular modeling which predicted that the low energy conformation of the spirocyclic structure **5** mimics the bioactive conformation of **3**.

The general synthetic route used to prepare benzotriazepine **4** and its analogs is outlined in Scheme 1. The strategy begins with reaction of previously described phenylenediamine **6** with phenyl isocyanatoformate to give the monosubstituted triazepine **7**.[12] This reaction proved to be low yielding and, under forcing conditions, the dominant product was the corresponding imidazolone, which control experiments showed was formed by heating compound **7** under the reaction conditions. Selective arylation of the triazepine scaffold to give compound **8** could only be achieved by a modification of a literature procedure utilizing triphenylbismuth, pyridine and copper(II) acetate.[13] Key to the success of our modification was that the reaction was performed in dioxane and at higher temperature (80 °C). Arylation of the remaining free N-H group to give compound **4** could only be achieved under conditions identical to those used for the transformation of compound **7**–**8**. Indeed, if an excess of triphenylbismuth and copper(II) acetate was used, arylation of both nitrogen atoms of compound **7** occurred and compound **4** could be isolated directly. Synthesis of analogs of compound **4** relied on preparation of novel triaryl bismuth reagents.[12] This was accomplished by conversion of the appropriate aryl bromide **9** to their corresponding Grignard reagents **10**, followed by quenching with $BiCl_3$ to give the required triarylbismuth reagent **11** (Scheme 1). Generally, the overall yield for this process was low (10–40% after chromatographic purification). However, the arylation of compound **8** to give the required intermediates was very efficient. With the fully substituted triazepines (**12**) in hand, progression to inhibitors **14**–**22** followed routine deprotection and functional group manipulations.

Synthesis of the spirocycle **5** and its analogs was more challenging. The sequence relied on the use of complex malonate derivatives encompased by the general structure **23**[12] (Scheme 2). These analogs were transformed to the seven-membered ring analogs using the strategy we have previously described[6,12] and then the N5 position was arylated to give compound **24**. Finally, the spirocyclic ring was formed through an intramolecular palladium-catalyzed malonate arylation under conditions developed in the accompanying letter to give compound **5** when X = H.[14] To prepare analogs of compound **5**, additional functionality was encorporated through the X-substituent to give the general structure **25**. This intermediate proved extremely versatile en route to spirocyclic analogs **26**–**31**.

The potency of compounds **3**, **4** and **5** is compared in Table 2, revealing that the three compounds were equipotent in the capsid assembly assay. Triazepine **4** showed a slight advantage over both benzodiazepine analogs **3** and **5** in terms of antiviral potency, but when taken together, the results indicated that both modifications were viable solutions to the lability of the C-3 stereocenter. In order to follow up on these results, we focused on developing new structural–activity relationships (SAR) by modification of the C3 or N3 phenyl substituent.

Simple substitution around the N3-phenyl ring of the triazepine series led to improvements in intrinsic potency. For example, simple substitution of a *m*-OMe or *p*-OMe group resulted in a threefold improvement in IC_{50} value (Table 3). Similar improvements were obtained with other small substituents, as exemplified by the *p*-OH and *p*-Me analogs **16** and **17** or the amido substituted analogs **18** and **19**. Similar to what was previously shown,[6] a wide range of

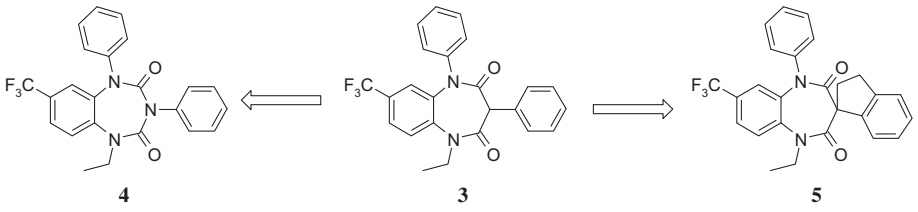
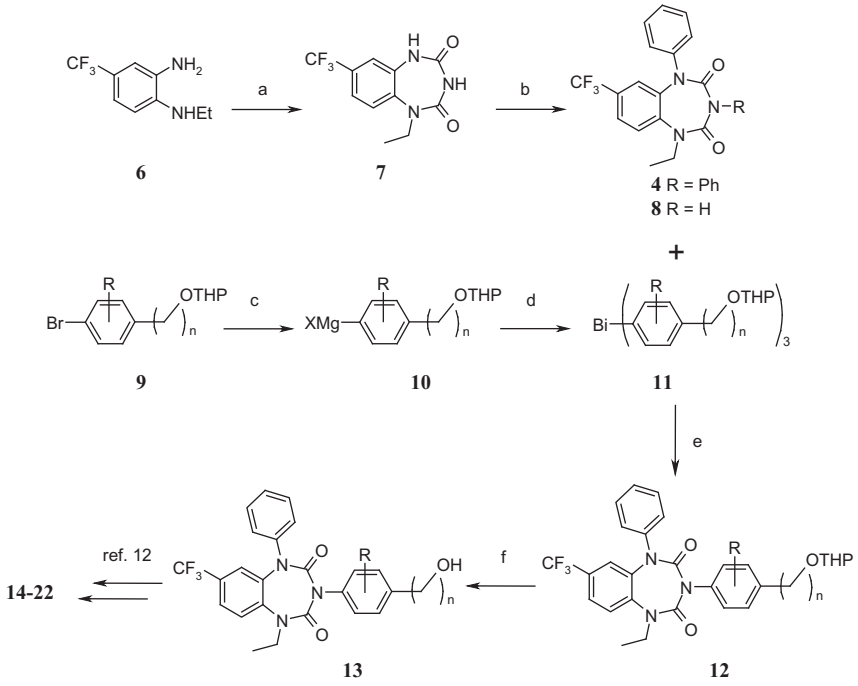
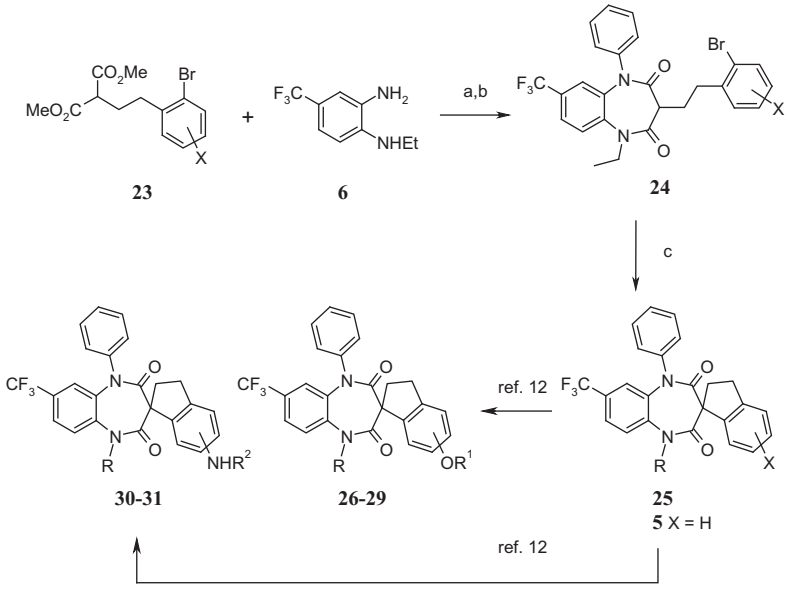


Figure 2. Modification of the 3-position of compound **1**.



Scheme 1. Reagents and Conditions: (a) PhO–CO–N=C=O, D, 26%; (b) Ph_3Bi, $Cu(OAc)_2$, pyridine, 1,4-dioxane D, 46%; (c) Mg(0), cat. I_2, D, (d) $BiCl_3$, THF, (e) **8** + **11**, $Cu(OAc)_2$, pyr, D; (f) H+, H_2O



Scheme 2. Reagents and Conditions: (a) (i) μW @ 170 °C, 52%, (ii) LiHMDS, THF, 21%; (b) Ph_3Bi, $Cu(OAc)_2$, Et_3N, DCM, 85%; (c) $[P(t\text{-}Bu)_3P]_2Pd$, Cs_2CO_3, THF, 80 °C, 70%.

Table 2
Comparison of compounds **3**, **4** and **5**

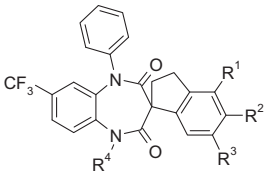
	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
3	0.73	2.6	—
4	0.63	0.6	>4.0
5	0.46	1.6	>16

functionality and lipophilicity was well tolerated in this region of the molecule, consistent with our X-ray crystallographic data suggesting that this pharmacophore protruded from the pocket of CA_{NTD} into the bulk solvent. In further support of this, increasing the size and polarity of the *m*-amido group to the morpholine analog **20** resulted in no change in potency. However, multiple substitutions of the phenyl group resulted in increases in the antiviral potency of the molecules, bringing the antiviral potency into the 100 nM range in the case of trisubstituted analog **22**.

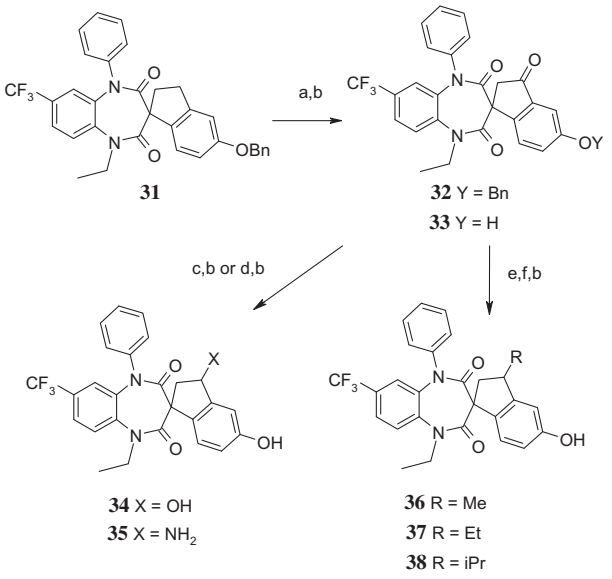
A similar exploration of the spirocyclic series was undertaken. In this case, the two positions *meta*- to the C3–C(aryl) bond are rendered non-equivalent as a consequence of the cyclization from one ortho position to C3 of the benzodiazepine. In the case of a hydroxyl substituent, walking this group around the phenyl ring illustrated that most substitutions were well tolerated, improving the intrinsic and antiviral potency by about twofold for the positions *ortho*- or *meta*- to the benzylic methylene of the cyclopentane ring (Table 4). Substituting the R^2 = OH group for an amino or methoxygroup was also well tolerated. The *p*-amino group was also used to introduce amide substituents, exemplified by proline derivative **31**, which showed similar antiviral potency to aniline **30**.

Addition of the spirocyclic ring also allowed exploration of structure–activity relationships not accessible in the original benzodiazepine or benzotriazepine lead series. Substitution of the cyclopentane ring was accomplished as shown in Scheme 3 and relied on facile functionalization of the benzilic postion through CrO_3 mediated oxidation to ketone **33**.[12] This ketone could then be modified in a number of ways to give inhibitors **34**–**38**. Overall (Table 5), we found that introduction of polar substituents at this position was poorly tolerated (cf. compounds **33**–**35** and compound **28**). Addition of a simple methyl group at the benzylic position was possible as compound **36** and **27** were equipotent, but as the size of this alkyl group increased, potency also decreased (cf. compounds **36**–**38** and compound **27**). Taken together, the results indicated that substitution on the cyclopentane ring was poorly tolerated and so further modifications were not pursued.

Table 4
Structure–activity relationships for analogs of compound **5**

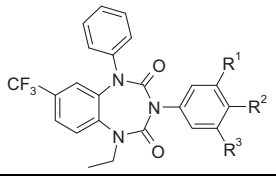


	R^1	R^2	R^3	R^4	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
26	OH	H	H	Et	0.24	0.78	>21
27	H	OH	H	Et	0.21	0.69	32
28	H	H	OH	Et	0.59	1.2	>27
29	H	OMe	H	Et	0.16	0.51	>4
30	H	NH_2	H	Me	0.16	0.41	>15
31	H	NH-Pro	H	Me	0.11	0.52	11



Scheme 3. Synthesis of analogs **33**–**38**. Reagents and Conditions: (a) CrO_3; (b) H_2, Pd–C; (c) $NaBH_4$; (d) $BnNH_2$, pTsOH, (e) RMgBr; (f) TFA/DCM.

Table 3
Structure–activity relationships for analogs of compound **4**



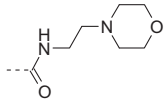
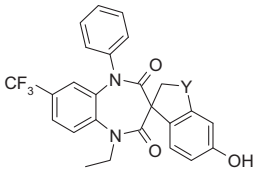
	R^1	R^2	R^3	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
14	OMe	H	H	0.21	1.2	>20
15	H	OMe	H	0.18	0.72	>16
16	H	OH	H	0.05	0.38	35
17	H	Me	H	0.19	0.54	>6.5
18	–CO–NHMe	H	H	0.28	1.2	>22
19	H	–CO–NHMe	H	0.13	0.48	31
20		H	H	0.22	1.1	>19
21	Me	OH	H	0.09	0.26	17
22	Me	OH	Cl	0.06	0.15	>10

Table 5
Structure–activity relationships for analogs **33–38**



	–Y–	IC_{50} (μM)
33	–CO–	1.5
34	–CH(OH)–	2.1
35	–CH(NH_2)–	3.6
36	–CH(Me)–	0.29
37	–CH(Et)–	0.73
38	–CH(*i*-Pr)–	1.1

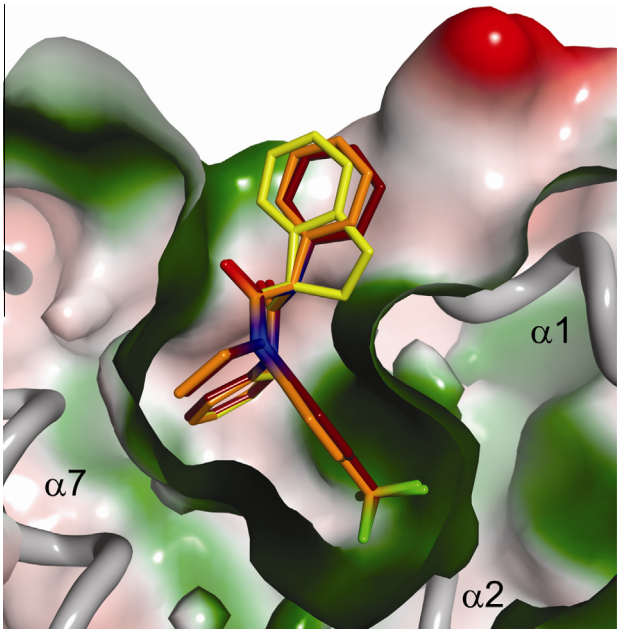


Figure 3. Superposed models of compounds **3** (orange), **4** (red) and **5** (yellow) bound to CA_{NTD}, based on related X-ray structures (PDBID 4E91). [7] The protein surface is coloured by hydrophobicity (green = hydrophobic, white = neutral, red = hydrophilic) and has been cut away to reveal the deeply bound compounds. The backbone trace of the protein is shown as a grey coil.

In order to further optimize the triazepine and spirobenzodiazepine series, we sought a structure-based approach to exploit information gleaned from SAR in the original 3-phenylbenzodiazepine series. Based on X-ray structures of related compounds bound to CA_{NTD}, molecular modeling of compounds **3**, **4**, and **5** confirms that all three analogs display similar trajectories from the RHS phenyl moiety (Fig. 3). The small, 10–15° variations among these trajectories may provide subtle but meaningful differences during optimization. In the following companion paper we disclose an extensive RHS exploration of the 3-phenylbenzodiazepine series represented by compound **3** and the transposition of this knowledge onto the newly described triazepine and spirobenzodiazepine series represented by compounds **4** and **5**, respectively.

In conclusion, we have designed replacements for the configurationally labile stereocenter at C3 of our 1,5-dihydrobenzo[*b*][1,4]diazepine-2,4-dione series of inhibitors of HIV-1 capsid assembly. Replacement of the C3 carbon with a nitrogen atom yielded the 1,5-dihydro-benzo[*f*][1,3,5]triazepine-2,4-dione analog **4**. Cyclization from the C3-position to the ortho position of the C3-phenyl group of compound **3** resulted in the spirocyclic 1,5-dihydrobenzo[*b*][1,4]diazepine-2,4-dione analog **5**. Both of the modifications were found to generate compounds that inhibit capsid assembly in a potency range comparable to that of lead compound **3**, but lacked a labile chiral center. Preliminary right-hand side SAR in both series indicated that improvements in potency are possible and a combination of molecular modeling and X-ray crystallographic data indicate that SAR might be transferable between the scaffolds represented by compounds **3**, **4** and **5**.

Acknowledgments

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9. IC_{50} and EC_{50} values are typically the mean of two determinations.
10. MMFF94x force field in Molecular Operating Environment (MOE); Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7.
11. The software Jaguar, version 7.0 (Schrödinger, LLC., New York, NY, 2007) was used for QM calculations (heteroatom pairs active; a Poisson Boltzmann model was used for solution calculations).
12. Detailed experimental procedures for most of the compounds in the present manuscript can be found in: Simoneau, B.; Deroy, P.; Fader, L.; Faucher, A.-M.; Gagnon, A.; Grand-Maitre, C.; Kawai, S.; Landry, S.; Mercier, J.-F.; Rancourt, J. PCT Int. Appl. WO 2011/100838.
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